Development of an efficient and flexible method for the solid-phase synthesis of N-hydroxypolyamine derivatives

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Abstract: Summary SUMMARY The most common aliphatic polyamines, spermine, spermidine and putrescine, are ubiquitous in all living organisms. Polyamines display a wide range of biological function in microorganisms as well as plant and animal systems. They play important roles in DNA stabilisation and modification, protein biosynthesis, the modification of neuroreceptors and their associated ion channels in mammalian central nervous system. Because polyamines are considered as therapeutic leads for the treatment of a variety of brain disor- ders such as Parkinson's and Alzheimer's diseases, it is well understood why new and efficient methods for their synthesis are being sought. A number of natural products, however, are additionally derivatised at the internal N-atom, like, the N-hydroxylated spider toxins Agel 448 and Agel 452 present in the venom of the spider Agelenopsis aperta (Figure 1). OH H OH H N N N N NH2 O H HN Agel 448 O H N NH2 N N N N H OH H H HO Agel 452 Figure 1. Representatives of N-hydroxylated polyamine spider toxins from Agelenopsis aperta. The chemical composition of spider venoms is heterogeneous. Two major classes of compounds are found, complex peptides and lower molecular weight molecules such as acylpolyamines. The latters are known to provoke a fast but reversible paralysis of the prey. Our group has contributed to the elucidation of the structure of many polyamine derivatives by high- performance liquid chromatography coupled with mass spectrometry (HPLC- MS) and tandem mass spectrometry (MS/MS or MSn) present in the venom of, e.g., Agelenopsis aperta, Paracoelotes birulai, Hololena curta and Larinioides folium. 1 Summary N-hydroxylated polyamine derivatives were found to decompose during the ionization process of liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) experiments. Whereas liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI- MS) showed a single peak for Agel 489, LC-APCI-MS showed two, with a difference of 16 Da (Figure 2). This observation can be explained by the reduction of the NOH- to the NH- group. Unfortunately no literature was found to confirm or discard this hypothesis. H OH H H N N N N N NH2 O H HN Agel 489 IndAc-3(OH)3343 APCI-MS ESI-MS Figure 2. APCI- and ESI-MS of acyl polyamine IndAc3(OH)3343. In connection with our ongoing studies in the analysis and synthesis of spider toxins in addition to our intention to understand the reductive behaviour during MS measurements, we were interested in extending our solid-phase strategy for the preparation of N-hydroxylated polyamine derivatives. Until now our method has been applied to the preparation of terminally acylated polyamine natural products and cyclic tri- and tetraamine derivatives. Poor yields obtained with the usual cleaving method, using ACE-Cl followed by hydrolysis with MeOH, led us to the introduction of a phenethyl bromide linker in-between the Merrifield support and the polyamine in elongation. We were confident that the Cope elimination provided an efficient transformation for the concurrent introduction of the desired 2 Summary N-hydroxy functionality and the cleavage of the final product from the resin (Scheme 1). Introduction Derivatisation H Br N Cl R1 of the linker of the resin Merrifield resin H R3 O N 4 R Elongation O Cope Elimination OH R3 R2 Oxidative Synthetic N N 4 N 1 R3 R4 R R cleavage transformations Scheme 1. The concept. This thesis presents the efficient synthesis of orthogonally protected N-hydroxylated linear tri-, tetra-, penta- and hexaamines on the Merrifield resin. It is shown that the approach for the preparation of the polyamine backbones on solid-support is flexible, allowing the contruction of the resin- bound polyamine portion by (1) reductive amination with a protected amino aldehyde (Scheme 2, orange), (2) by nucleophilic substitution with mono-pro-

tected diamines (Scheme 2, blue), with disulfonamides (Scheme 2, brown), but also (3) by reverse nucleophilic substitution with a protected aminobromide derivative (Scheme 2, orange/green) or with dibromo alkane compounds (Scheme 2, magenta). We thus laid the basis for the solid-phase synthesis of any N-hydroxylated polyamine derivatives. Ns, Boc, Alloc and Phth protective groups were demonstrated to be compatible with the oxidative procedure. Selective removal of the latter protective groups and insertion of the acyl moiety were performed on solid- support in-between the oxidation and the Cope elimination in order to keep the regioselectivity of the oxidation under control and give access to natural products such as Agel 395 and Agel 432 (Figure 3). 3 Summary HO OH H H N N N N NH2 O H Agel 395 OH H H N N N N NH2 O H HN Agel 432 Figure 3. Two N-hydroxy acylpentaamine natural products. The phenomenom observed during MS measurements was studied with a model compound, a synthetic N-hydroxylated tetraamine derivative. It was found that the reduction, oxidation and water elimination occurred during APCI to generate the corresponding amine, N-oxide, and imine. The investigation further revealed that the decomposition of hydroxylamine during APCI depends upon the concentration of the analyte and on the acidity of the solution introduced into the ionization source. The pH-dependence of decom- position was utilized for the development of an MS method that allows for the unambiguous identification of N-OH fuctionalities. This method was applied for the study of natural products including polyamine toxins from the venom of the spider Agelenopsis aperta and mayfolin, a cyclic polyamine derivative of the shrub Maytenus buxifolia. 4 NO2 O S O O O H NHR N N N N N N n m R' OH OH O O Ns Phth n = 1, m = 1 R Boc R' Boc and Alloc Alloc n = 0, m = 2 N-OH-Triamine Derivatives N-OH-Tetraamine Derivatives 4 steps 5 steps Br 6 steps 6 steps Summary N-OH-Pentaamine Derivatives N-OH-Hexaamine Derivatives O2N O2N O Scheme 2. The backbones preparation. Me O O S O Me O O S O H Me Me O NO2 N N N N Me O N N N Me O N N N N S H O H O S O O S O O OH O S O OH NO2 O2 N NO2 Reductive Amination, Protected Amino Aldehyde or Nucleophilic Substitution, Dibromo Alcane Nucleophilic Substitution, Protected Amino Bromide Nucleophilic Substitution, Mono Protected Diamine Nucleophilic Substitution, Disulfonamide Nucleophilic Substitution, Protected Amino Bromide Cope Elimination 5 ZUSAMMENFASSUNG Die am weitesten verbreiteten aliphatischen Polyamine Spermin, Spermidin und Putrescin sind allgegenwärtig in lebenden Organismen. Polyamine üben eine Vielzahl von biologischen Funktionen in Mikroorganismen sowie in Pflanzen und Tieren aus. Sie tragen zur Stabilisierung und Modifizierung von DNA bei und spielen eine wichtige Rolle bei der Biosynthese von Proteinen, der Modifizierung von Neurorezeptoren und deren zugehörigen Ionen- kanälen im Zentralnervensystem von Säugetieren. Da Polyamine als therapeu- tische Ansätze für die Behandlung von verschiedenen Hirnleistungsstörungen wie Parkinson oder Alzheimer berücksichtigt werden, ist es leicht nachvoll- ziehbar, dass neue und effizientere Methoden für deren Synthese gesucht werden. Etliche der Naturstoffe sind an einem internen N-Atom derivatisiert, wie die N-hydroxylierten Spinnengifte Agel 448 und Agel 452 der Spinne Agelenopsis aperta (Figur 1). OH H OH H N N N N NH2 O H HN Agel 448 O H N NH2 N N N N H OH H H HO Agel 452 Figur 1. Vertreter der N-hydroxilierten Polyamin-Spinnengifte von Agelenopsis aperta. Die chemische Zusammensetzung der Spinnengifte ist heterogen. Zwei Hauptklassen von Substanzen liegen vor: komplexe Peptide und Moleküle mit niedrigem Molekulargewicht wie Acylpolyamine. Letzere sind dafür bekannt, dass sie eine schnelle, aber reversible Lähmung der Beute hervorrufen. Unsere Gruppe hat zur Aufklärung von Strukturen vieler Polyamin-Derivaten aus dem Gift von Agelenopsis aperta, Paracoelotes birulai, Hololena curta und Larinioides folium mittels HPLC-MS (high performance liquid chromatography mass spectrometry) und MS/MS (tandem mass spectrometry) beigetragen. 6 Es wurde beobachtet, dass N-hydroxylierte Polyamine in dem Ionisierungsprozess während LC-APCI-MS (liquid chromatography/ atmos- pheric pressure chemical ionization-mass spectrometry) Messungen zerfallen. Mittels LC-ESI-MS (liquid chromatography/electronenspray ionization-mass spectrometry) wurde für Agel 489 wie erwartet ein Peak erhalten, wohingegen APCI-MS Analysen zwei Peaks mit einem Massenunterschied von 16 Da hervorbrachten (Figure 2). Diese Beobachtung lässt sich durch die Reduktion von NOH zu NH erklären. Allerdings wurde keine Literatur zur Stützung oder Widerlegung dieser Hypothese gefunden. H OH H H N N N N N N NH2 O H HN Agel 489 IndAc-3(OH)3343 APCI-MS ESI-MS Figur 2. APCI- und ESI-MS Spektren des Acylpolyamins IndAc3(OH)3343. Bis zu diesem Zeitpunkt wurden mit unserer Methode terminal acylierte Polyaminnaturstoffe und Cyklische Tri- und Tetraamin-Derivate synthetisiert. Zusammenhängend mit den laufenden Untersuchungen, der Analyse und Synthese der Spinnengiftkomponenten, weiteten wir unsere Strategie der Fest- phasen-Synthese auf N-hydroxylierten Polyamin-Derivate mit dem Ziel aus, das reduktive Verhalten während der MS Messungen zu verstehen. Beim Gebrauch der üblichen Methode zur Abspaltung der Moleküle von der Festphase mit ACE-Cl, mit anschließender Methanolyse, wurden un- genügende Ausbeuten erhalten. Dies veranlasste uns zur Einführung eines Phenethyl-bromid Linkers zwischen dem Merrifield Harz und den zu 7 verlängernden Polyaminen. Wir waren zuversichtlich, dass die Cope Eliminierung eine effiziente Umwandlung zur gewünschten N-Hydroxy- Funktionalität und die gleichzeitige Abspaltung des Produktes vom Harz gewährleisten würde (Schema 1). Einführung Derivatisierung H Br N Cl R1 Merrifield Harzes des Linkers des Harzes H R3 O N 4 R Verlängerung O Cope Eliminierung OH R3 R2 Oxidative Synthetische

N N 4 N 1 R3 R4 R R Abspaltung Umwandlungen Schema 1. Das Konzept. Diese Arbeit beschreibt eine effiziente Methode zur Herstellung von orthogonal geschützten, N-hydroxilierten, linearen Tri-, Tetra-, Penta- and Hexaamine am Merrifield-Harz. Wir zeigen, dass der Ansatz Polyamin- Strukturen auf der Festphase zu synthetisieren flexibel ist, weil er den sequentiellen Aufbau des Polyaminrückrates durch (1) reduktive Aminierung mit geschützten Aminoaldehyden (Schema 2, orange), (2) durch nukleophile Substitution mit einfach geschützten Diaminen (Schema 2, blau) oder Disulfonamiden (Schema 2, braun), (3) aber auch durch invertierte nukleo- phile Substitution mit geschützten Aminobromid-Derivaten (Schema 2, orange/grün) oder mittels Dibromoalkanen (Schema 2, magenta) ermöglicht. Mit dieser Arbeit wurde die Basis für die Festphasen Synthese praktisch beliebigen N-hydroxilierter Polyamin-Derivate gelegt. Wir haben gezeigt, dass Ns, Boc, Alloc und Phth Schutzgruppen kompatibel mit der oxidativen Prozedur der Abspaltung des Endproduktes sind. Die selektive Entfernung der genannten Schutzgruppen und die Einführung eines 8 Acyl-Rests wurden auf der Festphase, zwischen der Oxidation eines Amins zum entsprechenden N-Oxides und einen Cope Eliminierung, durchgeführt, um einerseits die Regioselektivität der Oxidation zu kontrollieren und andererseits den Zugang zu Naturstoffen wie z. B. Agel 395 und Agel 432 (Figur 3) zu erhalten. HO OH H H N N N N NH2 O H Agel 395 OH H H N N N N NH2 O H HN Agel 432 Figur 3. Zwei N-hydroxyacylpentaamin-Naturstoffe. Die Untersuchung von Phänomenen, die während APCI-MS-Messungen aufgetretenen sind, wurden mittels einer synthetischen N-hydroxylierten Modellverbindung durchgeführt. Sie zeigten, dass bei der APCI von N-hydroxylierten Verbindungen Reduktion, Oxidation und Wasserelimination ablaufen und dass die entsprechenden Amine, N-Oxide und Imine gebildet werden. Dabei hängt die Zersetzung der Hydroxylamine von der Konzentration des Analyten sowie der Acidität der durch die Ionenquelle eingesprühten Lösung ab. Diese pH-Abhängigkeit wurde genutzt, um eine MS-Methode zu entwickeln, welche die eindeutige Identifikation von N-OH Funktionalitäten erlaubt. Diese Methode wurde für weitere Studien an Naturstoffen wie den Polyaminen des Spinnengiftes von Agelenopsis aperta und dem Cyklischen Spermidin-Alkaloid Mayfolin der Pflanze Mayetenus bruxifolia angewandt. 9 10 NO2 O S O O O H NHR N N N N N N n m R' OH OH O O Ns Phth n = 1, m = 1 R Boc R' Boc und Alloc Alloc n = 0, m = 2 N-OH-Triamin-Derivate N-OH-Tetraamin-Derivate 4 Stufen 5 Stufen Br 6 Stufen 6 Stufen N-OH-Pentaamin-Derivate N-OH-Hexaamin-Derivate O2N O2N O Me O O S O Me O O S O H Me Me Schema 2. Synthese des Polyamingerüsts. N N O NO2 N N Me O N N N Me O N N N N N S H O H O S O O S O O OH O S O OH NO2 O2N NO2 Reduktive Aminierung, Geschütztes Aminoaldehyde oder Nukleophile Substitution, Dibromoalkane Nukleophile Substitution, Geschütztes Aminobromid Nukleophile Substitution, Einfach Geschütztes Diamine Nukleophile Substitution, Disulfonamide Nukleophile Substitution, Geschütztes Aminobromid Cope Eliminierung

DEVELOPMENT OF AN EFFICIENT AND FLEXIBLE METHOD FOR THE SOLID-PHASE SYNTHESIS OF *N*-HYDROXYPOLYAMINE DERIVATIVES

DISSERTATION

zur

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La moindre chose contient un peu d'inconnu.
Trouvons-le.

Guy de Maupassant

Parce qu'il est parti beaucoup trop tôt…

TABLE OF CONTENT

PREFACE

This thesis consists of four separated chapters. Each chapter is independent from the others and has its own numbering of the schemes, figures and references.

Chapter 1 is an introduction to the natural polyamines and shows their properties. A short overview of the history of polyamine derivatives preparation on solid-support is presented.

Chapter 2 describes the development of a method for the solid-phase synthesis of *N*-hydroxypolyamine derivatives accompanied with « in-solution » studies for the design of a new linker. Investigations for the design of a new linker were performed by Dipl. Chem. *Denise Pauli* within her diploma work in our group. In this chapter the preparation of a small library of orthogonally protected tri- and tetraamine derivatives is shown. Part of the content of this chapter is already published in *Eur. J. Org. Chem.* **2008**, *33*, 5518–5525.

Chapter 3 presents the optimisation of the deprotection procedures with the preparation of « free » *N*-hydroxy tri- and tetraamines.

Chapter 4 consists in the application of the optimised procedures described in chapter 2 and 3 for the preparation of two natural products detected in the venom of the spider *Agelenopsis aperta*.

Chapter 5 exhibits the use of one of the synthetic *N*-hydroxypolyamine as a model compound for the investigations of the decomposition of such components during atmospheric pressure chemical ionization. The content of this chapter is already published in *J. Mass Spectrom.* **2010**, *45*, *2*, 190–197.

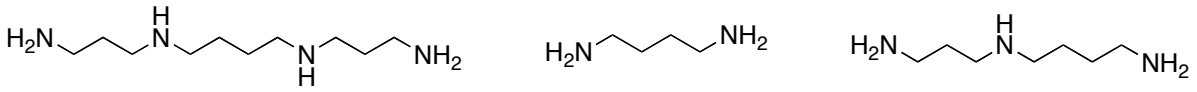
— Chapter 1 —

Polyamines in Nature — Spider Venom & Polyamine Toxins

1. POLYAMINES

1.1. Structure and Distribution

Aliphatic di- and polyamines are widely spread natural products found in microorganisms, plants as well as in animals. They often occur as conjugates to a number of different biological structures but also in free form (called biogenic amines). They are known since 1678, when *Lewenhoeck* (today written as *Leeuwenhoeck*), examined for the first time a linear tetraamine derivative obtained from sperm, spermine phosphate.[1] In addition to the almost ubiquitous spermine (**1**) and the two equally abundant compounds putrescine (**2**) and spermidine (**3**) (Figure 1), a great number of other biogenic polyamine compounds are known to date.



Spermine, **1** PA343 | Putrescine, **2** PA4 | Spermidine, **3** PA34

Figure 1. Most ubiquitous polyamines.

Other polyamines with less common structures are also distributed in the plant kingdom like norspermine (**4**) and norspermidine (**5**), initially isolated from thermophilic[2,3] and halophilic bacteria[4] but also found in algae[5] and mosses.[6] Homospermidine (**6**) was found in algae,[7] mosses[6] and ferns. Two uncommon polyamines occuring in higher plants are the diamine cadaverine (**7**) and the tetraamine canavalmine (**8**) (Figure 2). Cadaverine (**7**) is mainly

found in the Leguminosae[8,9]; canavalmine (**8**) was isolated from seeds of *Canavalia gladiata*.[10] It was shown that seeds of leguminous plants are a rich source of uncommon polyamines. For instance, a serie of linear and branched pentaamines, hexaamines, and heptaamines were found in the seeds of *C. gladiata*[11] and *Vicia sativa L.*[12]

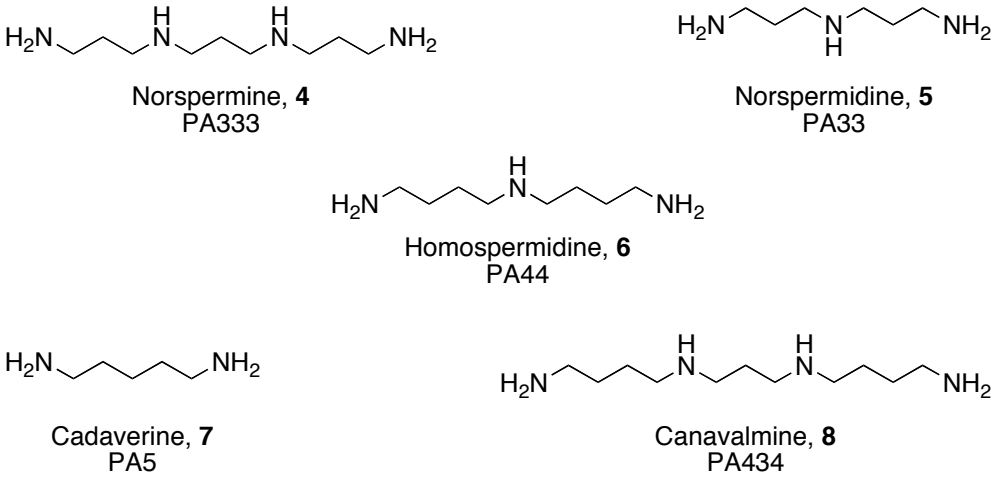


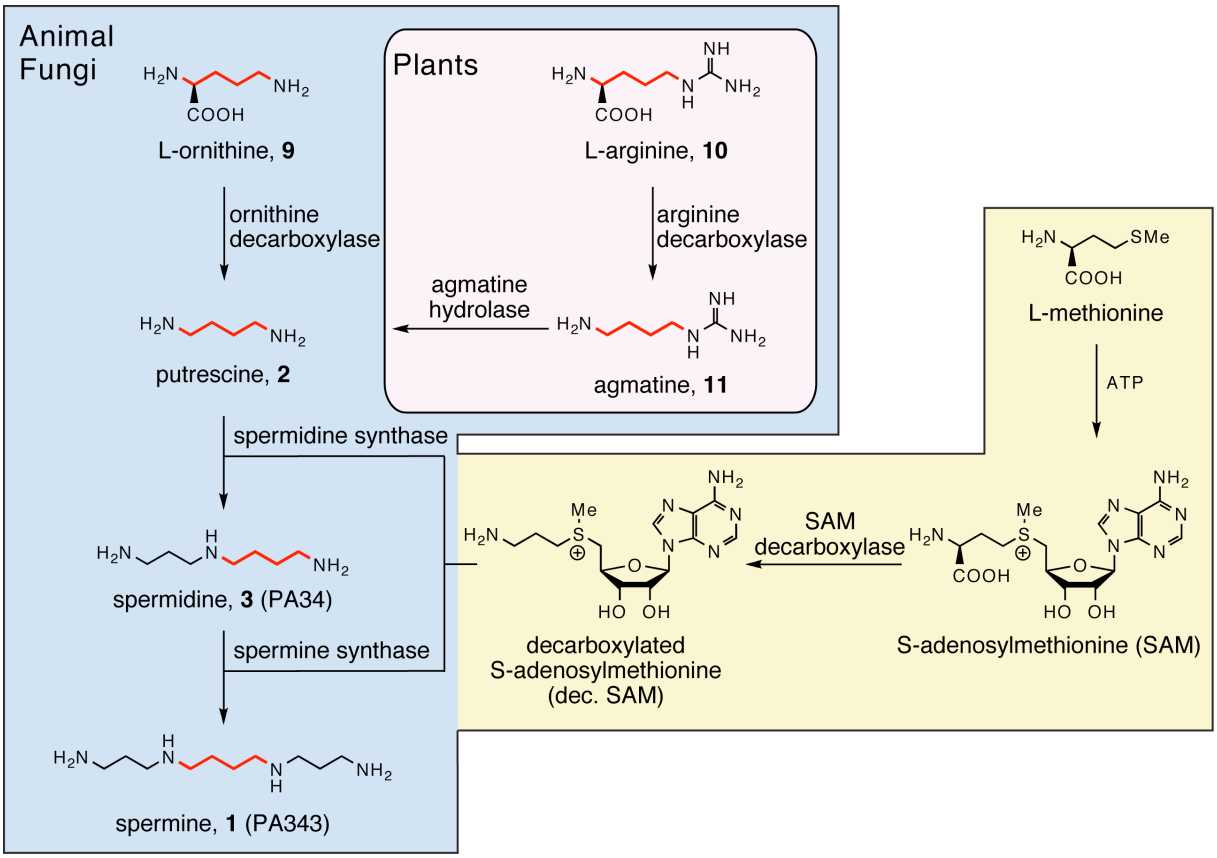
Figure 2. Other uncommon polyamines.

IUPAC systematic names are generally not used, and it is appropriate to use trivial names for these natural products. For the most abundant structures, the trivial names are adopted and well accepted in literature. Combinations of these trivial names with prefixes like nor-, homo-, and *sym*-homo for the several spermidine and spermine analogs are still in use, but such name, given without formula, might lead to confusion. Better suited with respect to clarity, and also applicable for and unusual polyamines is the « PA-nomenclature ». It abbreviates the linear polyamines by the prefix « PA » followed by the number of methylene units in-between the several N-atoms. For instance, PA4 stands for putrescine (**2**), PA34 for spermidine (**3**), and PA343 for spermine (**1**)[13] (Figure 1).

1.2. Biosynthesis of Polyamines

The biosynthesis of polyamines has been studied in several higher plants.[14-18] Putrescine (**2**), the precursor of most of the common polyamines, is

probably formed from L-ornithine (**9**) in all plants, animals, and micro-organisms by the action of the enzyme ornithine decarboxylase. Many plants and bacteria also possess an arginine decarboxylase, which allows the formation of putrescine (**2**) from L-arginine (**10**) *via* its decarboxylation product, agmatine (**11**). The enzyme converting L-arginine (**10**) to agmatine (**11**, obtained from leaves of barley seedlings) was partially characterised and purified in the 1960's.[14] The synthesis of spermidine (**3**) from putrescine (**2**) is controlled by spermidine synthase, which transfers an aminopropyl group from decarboxylated S-adenosylmethionine to a terminal amino group of putrescine (**2**). Spermine (**1**), finally, is obtained from spermidine (**3**) by spermine synthase, transfering selectively a second aminopropyl group to the second terminal amino group of the former putrescine (Scheme 1).



Scheme 1. Biosynthesis of putrescine, spermidine and spermine.

1.3. Polyamine Functions

Polyamines display a wide range of biological effects, and the last 30 years have witnessed an increasing research activity particularly in the field of their biological functions in microorganisms as well as plant and animal systems. In plants, this category of compounds is essential in cell growth and development.[19-21] Polyamines can interact strongly with nucleic acids and were found to play important roles in their biosynthesis and metabolism. Interactions between polyamines and DNA consists of electrostatic attractions between the polycationic polyamines and the negatively charged phosphate moieties of the DNA backbones.[22-24] This results not only in a stabilisation of the conformation[25-29] or in folding induction[30-32] of the DNA molecule but also in the protection of the DNA from denaturation caused by heat, chemicals and irradiations.[33] Polyamines are known to affect protein biosynthesis, but also to be involved in the differentiation of mammalian cells.[25,34,35] Increases of polyamine concentrations in cells have been linked to certain types of cancer, e.g., brain tumours.[36] Inhibition of polyamine biosynthesis in cultured cells by α-difluoromethylornithine, an inhibitor of the enzyme ornithine decarboxylase, causes a substantial depletion of intracellular putrescine and spermidine concentration, which was accompanied with an inhibition of cell proliferation. Polyamine derivatives were also shown to interact with ion channels in the mammalian central nervous system[37-42] (see 2.2.). This is the reason why polyamine derivatives are considered as therapeutic leads for the treatment of a variety of brain disorders such as *Parkinson*'s[43] and *Alzheimer*'s diseases.[44-47] Because of the manifold biological activities of polyamines, it is well understood that new and efficient methods for their synthesis are being sought.

2. SPIDER VENOM & POLYAMINE TOXINS

More than 30 000 spider species are known to date. They are grouped in 3618 genera and 110 families. Spiders are endowed with the capacity to manufacture chemicals which kill or paralyze their prey (mainly invertebrates,

especially insects). The most prominent class of components are polypeptides and proteins, which are neurotoxic, cytotoxic, or enzymatically active. Lower molecular weight molecules like polyamine derivatives are also found. These toxins are found to be neurotoxic, acting primarely on ion channels.[39,48-50]

2.1. General Structure and Nomenclature

Polyamine spider toxins have two structural particularities: all possess a linear α,ω-diamino polyazaalkane backbone (part C, Figure 3) modified at one end with a more or less lipophilic unit (or chromophore), in most of the cases an aromatic acyl group (part A, Figure 3). For some classes of toxins, the head portion A is separated from the polyamine backbone by one or more α-amino acid moieties (part B, Figure 3), and the most complex toxins are further modified at the tail of the polyamine backbone with an additional basic amino acid fragment (part D, Figure 3). The nomenclature of the polyamine toxins is not homogeneous throughout the literature. It is common to use abbreviations based on the genus of the spider from which they are derived and the molecular weight of the respective compounds, e.g., **Arg 636** (Figure 3). [13,50]

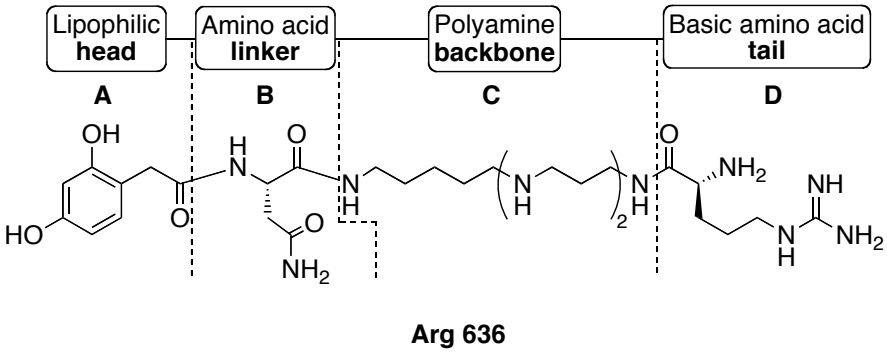


Figure 3. Structure of polyamine spider toxins illustrated by **Arg 636**.

2.2. Biological Activities of Polyamine Toxins

Polyamine toxins have neurotoxic effects and provoke a fast paralysis of the invertebrate prey. Acylpolyamines act as antagonists of ionotropic receptors by blocking, in a reversible manner, neuromuscular junctions and use as primarily targets the ionotropic glutamate receptors, e.g. the α-amino-3-

hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPAR), which mediate fast synaptic transmissions in the central nervous system. Polyamine toxins are unique as they selectively block Ca^{2+} permeable AMPARs, and recent studies[40] showed that they have to be considered more as a non-competitive than as competitive antagonists since it was found that their affinity for the closed channels was higher than for the open ones. The development of specific agonists and antagonists for ionotropic glutamate receptors has potential for the preparation of novel drugs for neurological, mental and psychiatric disorders. Since several health disorders arise as a consequence of alteration of the function of Na^+, K^+ and Ca^{2+} channels *via* glutamate receptors, and since acylpolyamines act as antagonists to such channels preventing ionic flux, spider acylpolyamines could find a role as novel agents used for the regulation of such ion channels.

2.3. The Spider Agelenopsis Aperta — Agelenidae Toxins

It is reasonable to classify the spider toxins according to their source and the animal family, since toxins found in different species of the same family are structurally closely related. We were interested in the identification of authentic components of the venom of the spider *Agelenopsis aperta*, *Araneomorphae* (modern spider) (Figure 6). *Agelenopsis aperta* is a large typical funnelweb spider found throughout most of southwestern USA, but also in the region of the Caucase in habitats as different as marine estruaries and desert dunes. The venom used for our investigations was obtained from *Fauna Laboratories*, *Ltd.*, in Almaty (Kazakhstan). The structures of the Agelenidae toxins consist of the lipophilic heads A and the polyamine backbones C, illustrated in Figure 4. Only four different chromophores were observed, two benzoic acid moieties (4-OH-Bz and 2,5-$(OH)_2$-Bz) and two indolacetic acid groups (IndAc and 4-OH-IndAc) (Figure 4).[51]

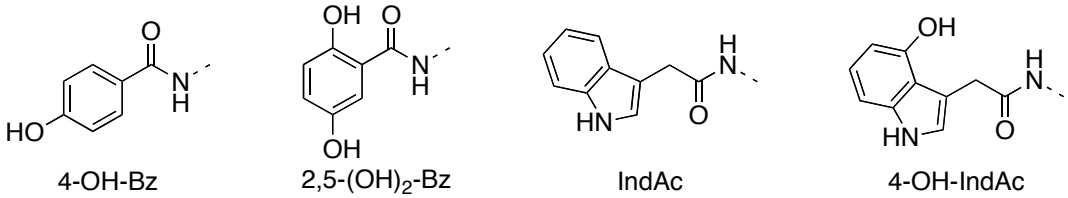


Figure 4. Lipophilic heads found in Agelenidae toxins.

The polyamine backbones of the Agenlenidae toxins are found to be particularly diverse : tetra-, penta- and hexaamines were discovered with variations in the skeleton by the succession of the oligomethylene units in-between the several N-atoms. In addition, a number of natural products are additionally derivatised at an internal N-atom, like the *N*-hydroxylated spider toxins **Agel 448** and **Agel 452** (Figure 5).[51]

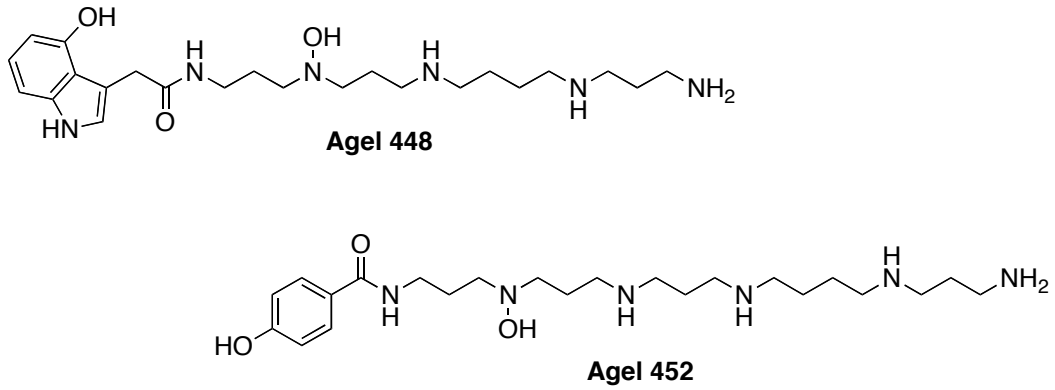


Figure 5. Representatives of *N*-hydroxylated polyamine spider toxins from *Agelenopsis aperta*.

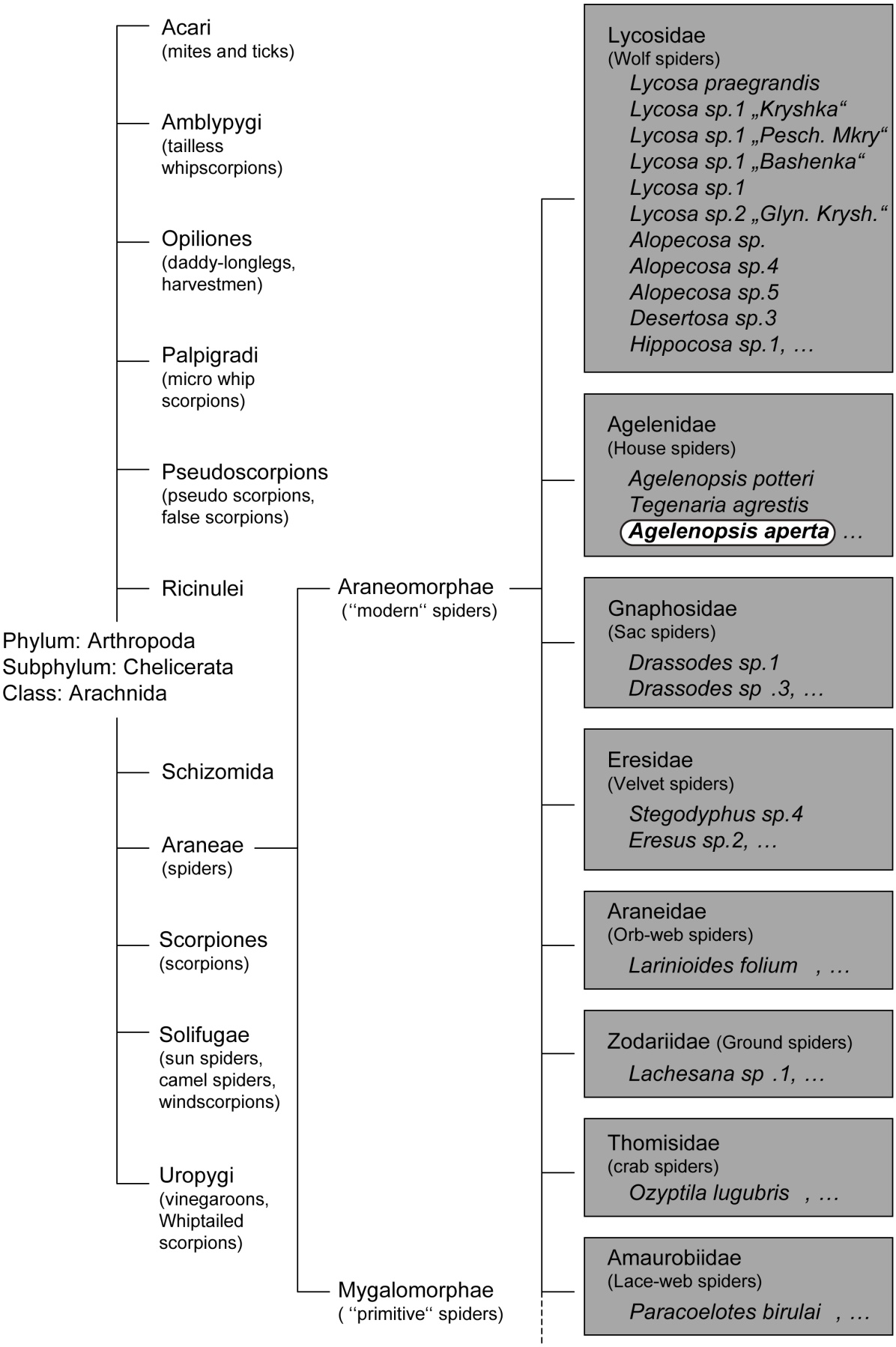


Figure 6. Phylogenetic tree.

3. Structural Elucidation of Polyamines by HPLC-MS and MS/MS

3.1. Composition of the Spider Venom

Spider venoms are composed of a number of different classes of biologically active compounds (Figure 7). They contain aminoacids, purine bases, peptides, proteins and free as well as acylated polyamines. The latter are present as trace amounts only in this complex mixtures, which is the reason why classical analytical methods are unsuitable for the identification and the characterisation of most of the polyamine spider toxins so that highly sensitive tools were required.

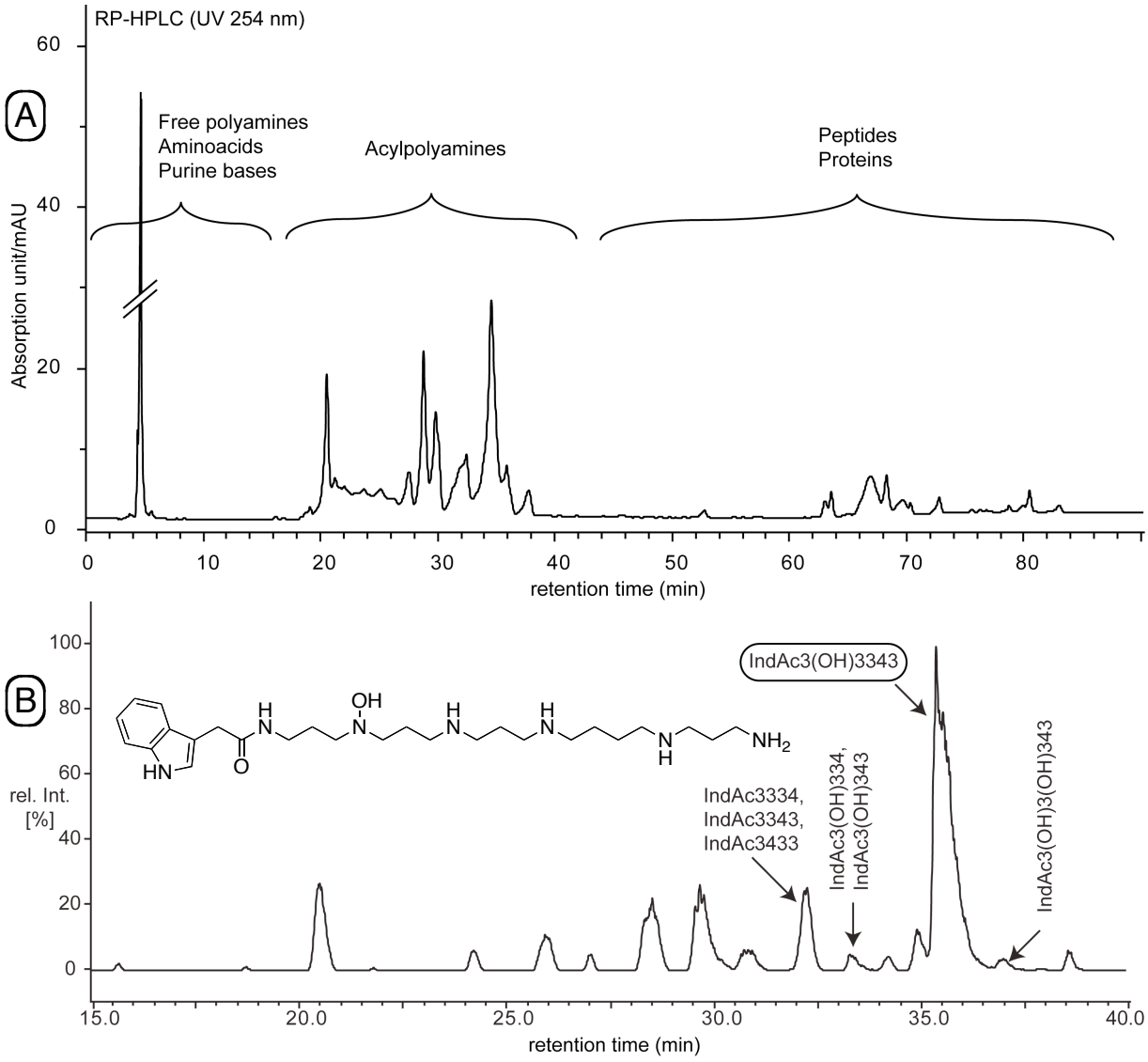


Figure 7. (A) HPLC of *Agelenopsis aperta* venom, (B) The region of the acylpolyamines.

The method of choice for the investigation of spider venoms is the mass spectrometry (MS) and particularly atmospheric pressure chemical ionization (APCI-MS). APCI-MS was chosen because it is a mild ionization method which produces quasi-molecular ions of the analyte with low fragmentation. Hence, the ions generated usually provide direct and unequivocal information about the mass of the sample molecule. Our group has contributed to the structural elucidation of many acylpolyamines — present in, e.g., *Agelenopsis aperta*,[51] *Paracoelotes birulai*,[52] *Hololena curta*,[52] *Larinioides folium*[53] and *Ozyptila lugubris*[54] — by the use of HPLC-MS and -MS/MS (Figure 8). Concerning the spider we are interested in, *Agelenopsis aperta*, four different aromatic acyl moieties and nine polyamine backbones were found (Figure 4).

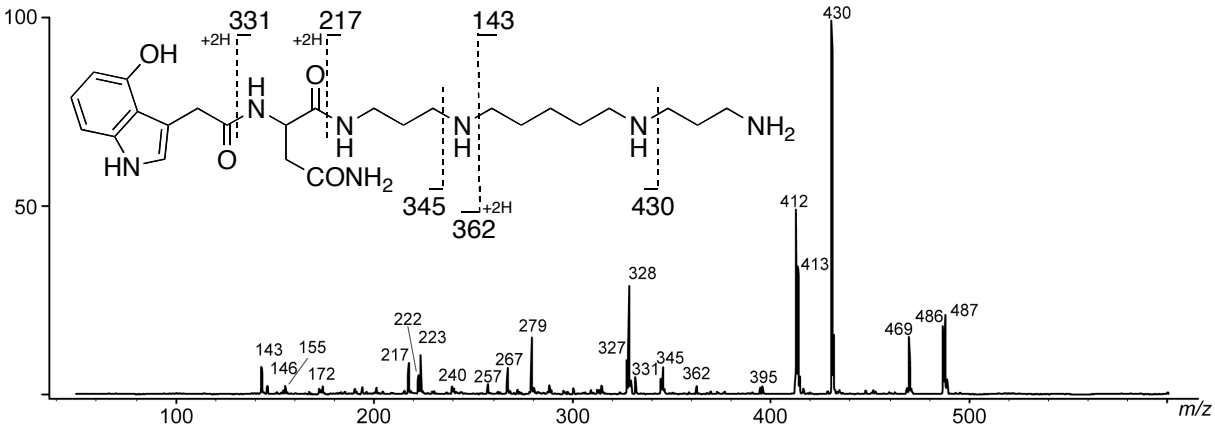


Figure 8. Structure elucidation of **LF 503B** by MS/MS.

3.2. APCI-MS vs. ESI-MS

In the course of our investigations of spider venoms,[51,55-57] in order to confirm the results obtained by APCI-MS experiments, the venom of *Agelenopsis aperta* was investigated performing electrospray ionization (ESI-MS) measurements. Unfortunately, the comparison of the two methods revealed some differences in the spectra. The HPLC-APCI-MS spectra showed, in the polyamine region, additional compounds but less abundant. The *m*/*z* values of these peaks were the same of already elucidated compounds. In addition, MS/MS were identical which meant that the polyamine backbones were the same. Nevertheless, since retention times were different, the components had

to be different. The first hypothesis was that they were isomers at the chromophore moiety due to the position of the hydroxyl group. The latter supposition was discarded by UV investigations. HPLC-ESI-MS chromatogram — under the same chromatographic conditions — revealed for a single HPLC peak the disappearance of these additional ions, demonstrating that there were not as many coeluting compounds as indicated in HPLC-APCI-MS. In addition, the missing ions always presented an *m*/*z* value with a difference of 16 or 32 Da (Figure 9).

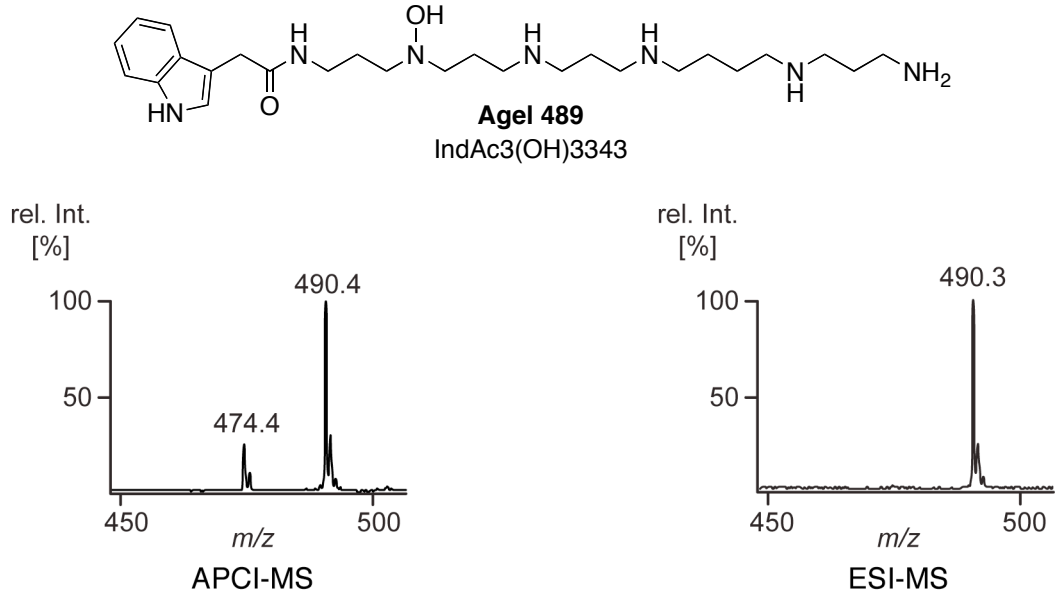


Figure 9. APCI- and ESI-MS of acylpolyamine IndAc3(OH)3343.

This can be explained by the reduction of one or two hydroxyl groups. Unfortunately no literature was found to confirm or discard this hypothesis. In particular with APCI-MS, for which the analyte solutions are typically heated to 300–400 °C prior to ionization, the risk of formation of artefacts can be expected to be relatively high since the elevated temperatures could lead to thermally induced decomposition. In fact decomposition reactions during APCI-MS were found, e.g., in MS investigations of aromatic nitro compounds,[58,59] *N*-oxides[60-64] and imines.[65] Upon APCI-MS, all three types of compounds were partially reduced to the corresponding amines.

4. SOLID-PHASE CHEMISTRY

4.1. History

Synthesis with the help of a solid-support is nowadays quite common and largely applied, particularly for the preparation of librairies of compounds by combinatorial chemistry. The history of solid-phase chemistry began half a century ago, in 1963,[66] when *Robert B. Merrifield* changed revolutionarily the synthetic approach for the preparation of peptides. *Robert B. Merrifield* in *Solid Phase Synthesis* (*Nobel Lecture*):[67] « *The plan was to assemble a peptide chain in a stepwise manner while it was attached at one end to a solid-support. With the growing chain covalently anchored to an insoluble matrix at all stages of the synthesis the pepitde would also be completely insoluble and, furthermore, would be in a suitable physical form to permit rapid filtration and whashing.* […] *Such a system offers four main advantages:*

- *It simplifies and accelerates the multistep synthesis because it is possible to carry out all the reactions in a single reaction vessel and thereby avoid the manipulations and attendant losses involved in the repeated transfer of material.*
- *It also avoids the large losses which normally are encountered during the isolation and purification of intermediates.*
- *It can result in high yields of final products through the use of excess reactants to force the individual reactions to completion.*
- *It increases solvation and decreases aggregation of the intermediate products.* »

The *Merrifield* resin is a polystyrene resin based on a copolymer of styrene and 1 % divinylbenzene as cross-linking agent (Figure 10). The resulting beads are derivatised with a chloromethyl group to allow the attachment of the starter of the synthesis. These days, peptide but also nucleic acid sequences are prepared with automated synthesizers.

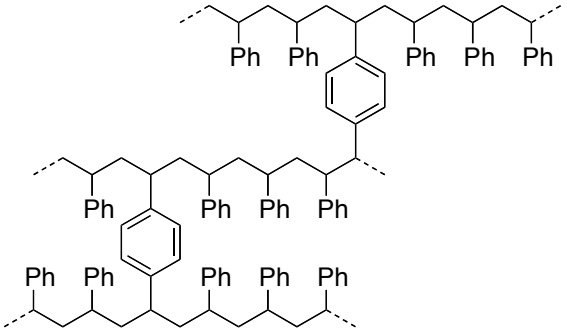
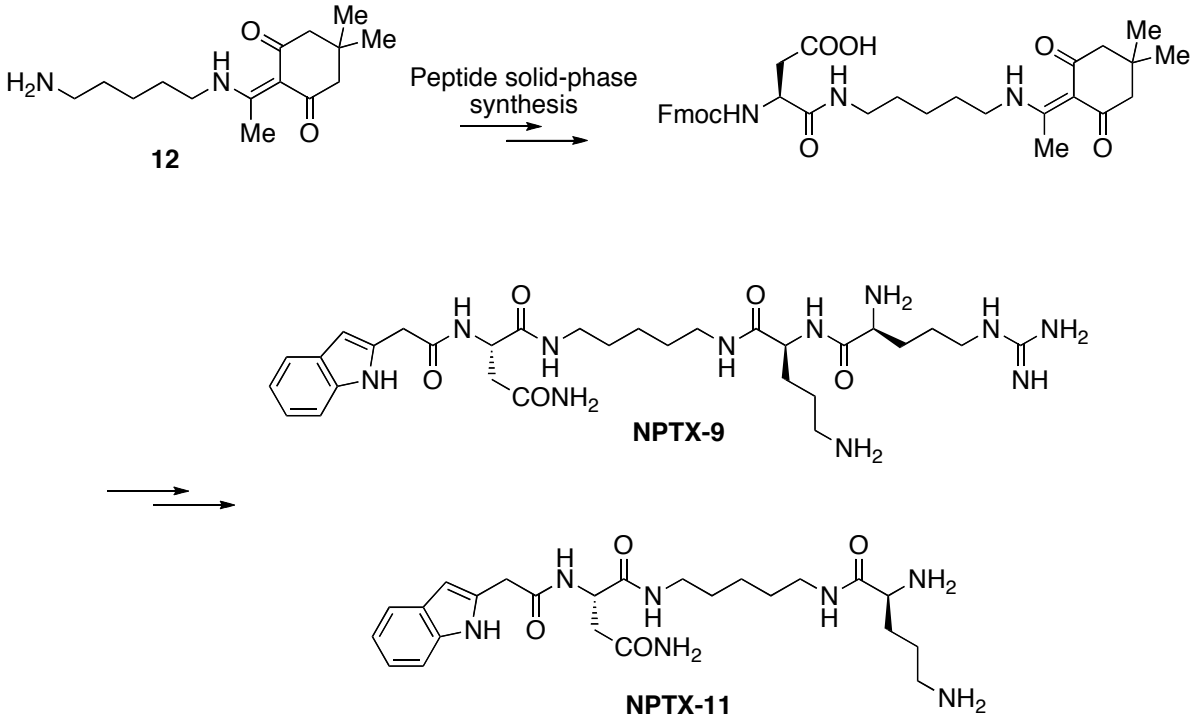


Figure 10. The *Merrifield* resin, a copolymer of styrene and divinylbenzene (1%).

Efficiency of the attachement and the removal of molecules to and from the polymeric resins relies on the appropiate choice of the linker group. The attachement point between the linker and the solid support has to be chemically inert during the synthesis and the cleavage. The combination of resin and linker can be considered as an insoluble and immobilizing protecting group for the molecule in preparation. The linker should be inert towards a wide range of reaction conditions but still allow the final product to be efficiently cleaved from the polymer in a very selective manner.

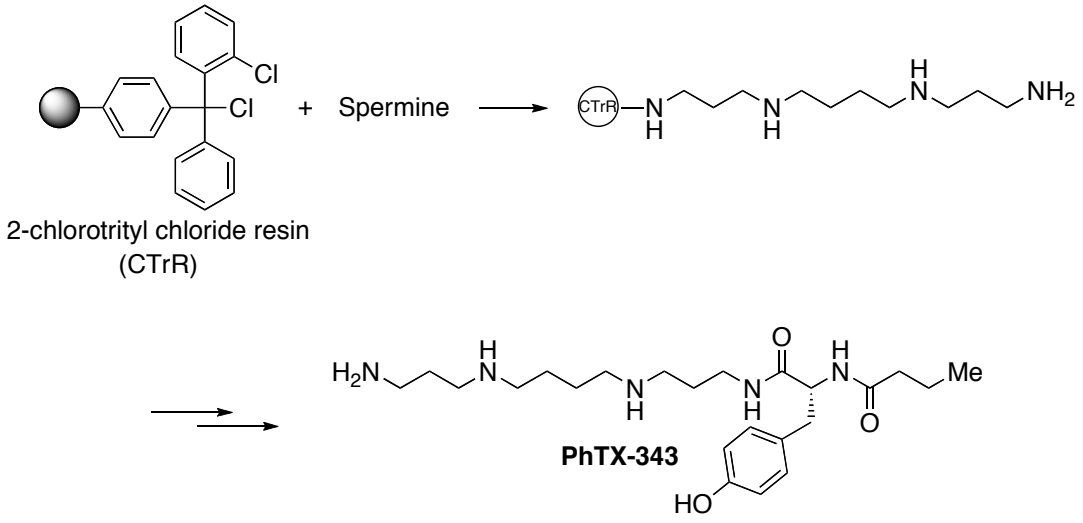
4.2. Synthesis of Polyamines on Solid-Support

The synthesis of polyamines in solution is a laborious task since it involves intensive use of protecting group strategies.[68-72] In addition to their good solubility in water, purification is often difficult due to the high polarity and basicity of the compounds. Solid-phase synthesis of polyamines and polyamine derivatives facilitates the procedure, since the work-up and purification operations are largely reduced to simple filtrations and washings of the resin. *Bycroft* et al. reported in 1994 one of the first synthesis of a polyamine with the help of solid-phase.[73] They succeeded in the preparation of the spider toxins Nephilatoxin-9 and -11, **NPTX-9** and **NPTX-11**, using a solid-supported synthesised peptide coupled with a cadaverine derivative **12** (Scheme 2).



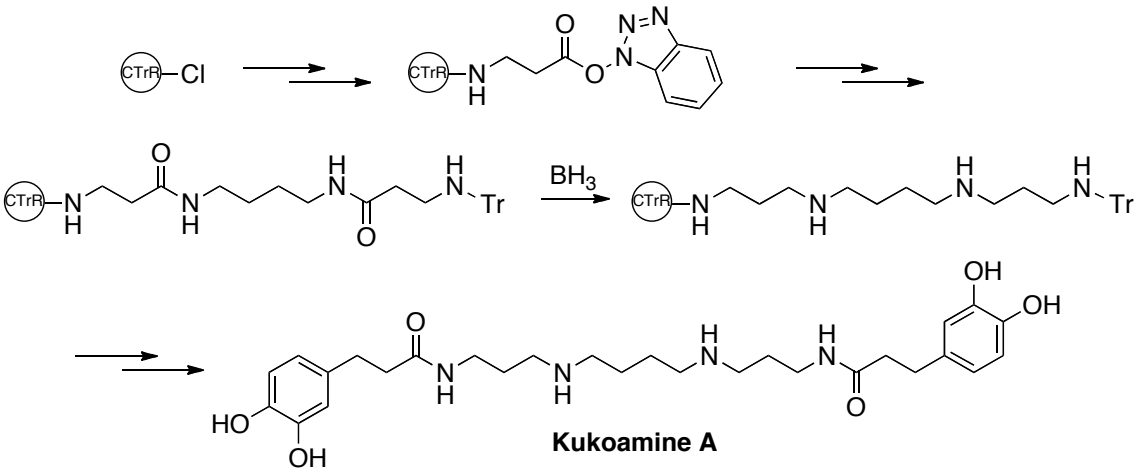
Scheme 2. Synthesis of **NPTX-9** and **-11** by *Bycroft* et al.

Two years later, the same team published the preparation of an analog of the wasp toxin philanthotoxin-433 (PhTX-433) from *Philanthus triangulum*, **PhTX-343**.[74] In this case, a 2-chlorotrityl chloride resin (CTrR) was used concurrently as protective group and polymer (Scheme 3).



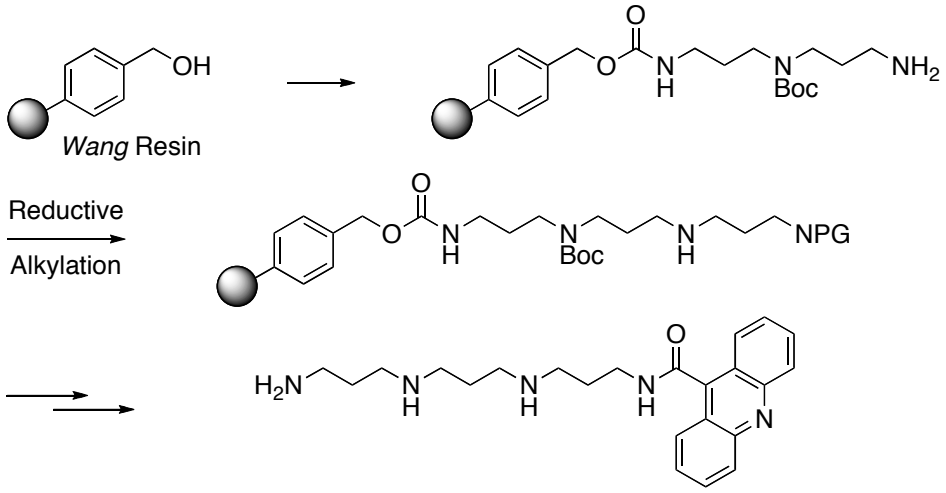
Scheme 3. Synthesis of **PhTX-343** by *Nash* et al.

The same year, *Marsh* and coworkers synthesised N^1,N^8-bis(glutathionyl)-spermidine (trypanothione) and generated a library of 576 analogs starting with the immobilisation of spermidine onto a solid-support followed by derivatisation with aminoacids.[75] In the meanwhile, the spermine alkaloid **Kukoamine A**, has been efficiently synthesised on 2-chlorotrityl resin by *Karigiannis* et al.[76] (Scheme 4). The procedure involved firstly, the formation of the carbon backbone framework of spermine by coupling putrescine to activated esters. Finally, the resulting amide groups were reduced using diborane.



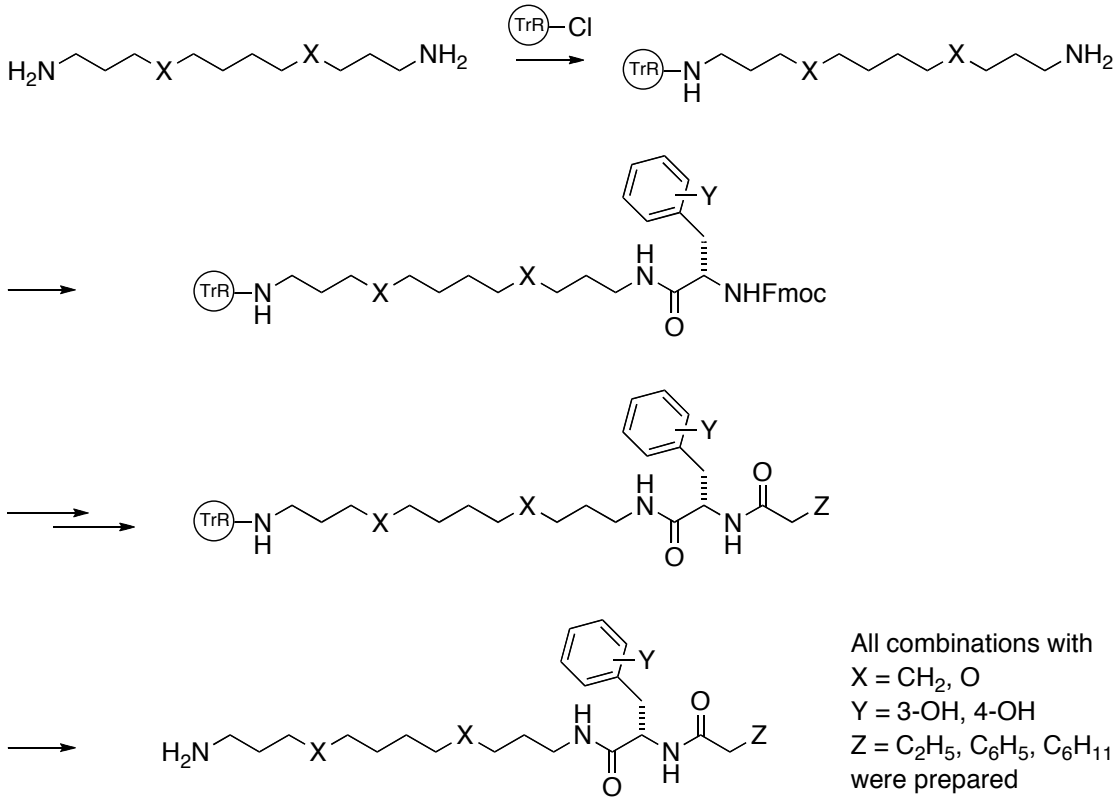
Scheme 4. Solid-phase synthesis of **Kukoamine A**.

In 1999, a novel reductive alkylation route to unsymmetrical tetraamine derivatives was introduced by *Carrington* et al.[77] The method consisted in using the *Wang* resin and an orthogonal protective group strategy followed by acylation of the terminally primary amine (Scheme 5).



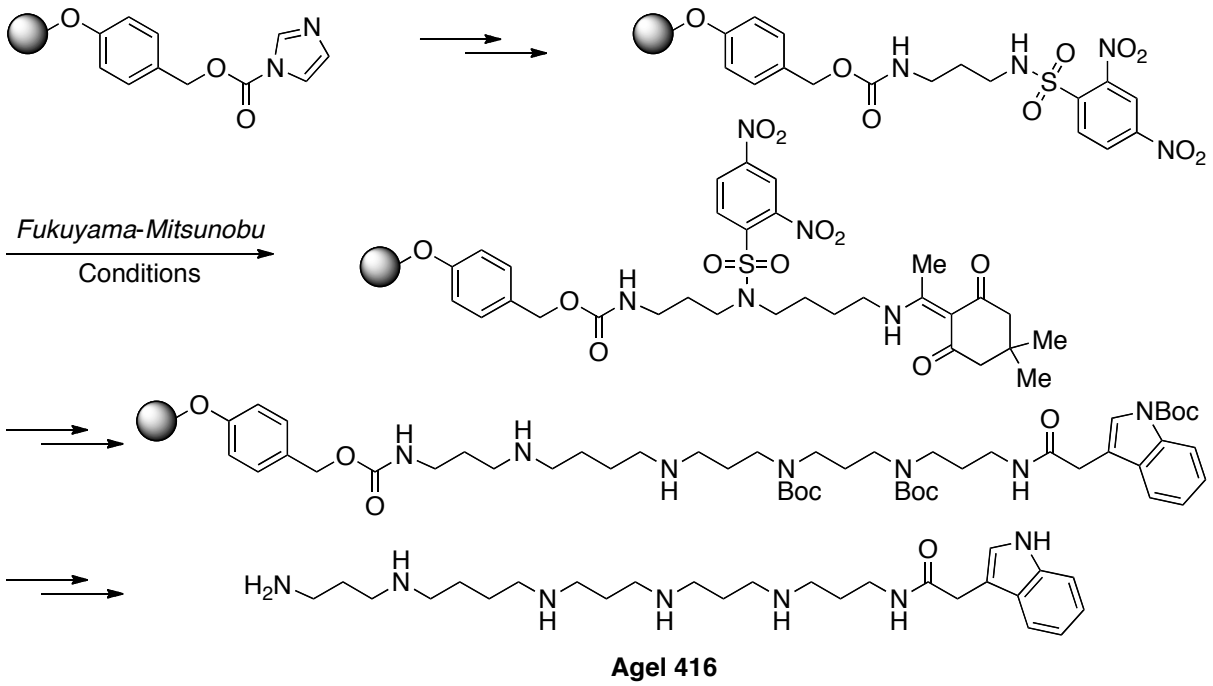
Scheme 5. Reductive alkylation on solid-phase.

A combinatorial library of Philanthotoxin analogs was reported in 2000 by Strømgaard[78] using the synthetic sequence acylation/deprotection/acylation starting with a trityl resin (TrR) (Scheme 6).



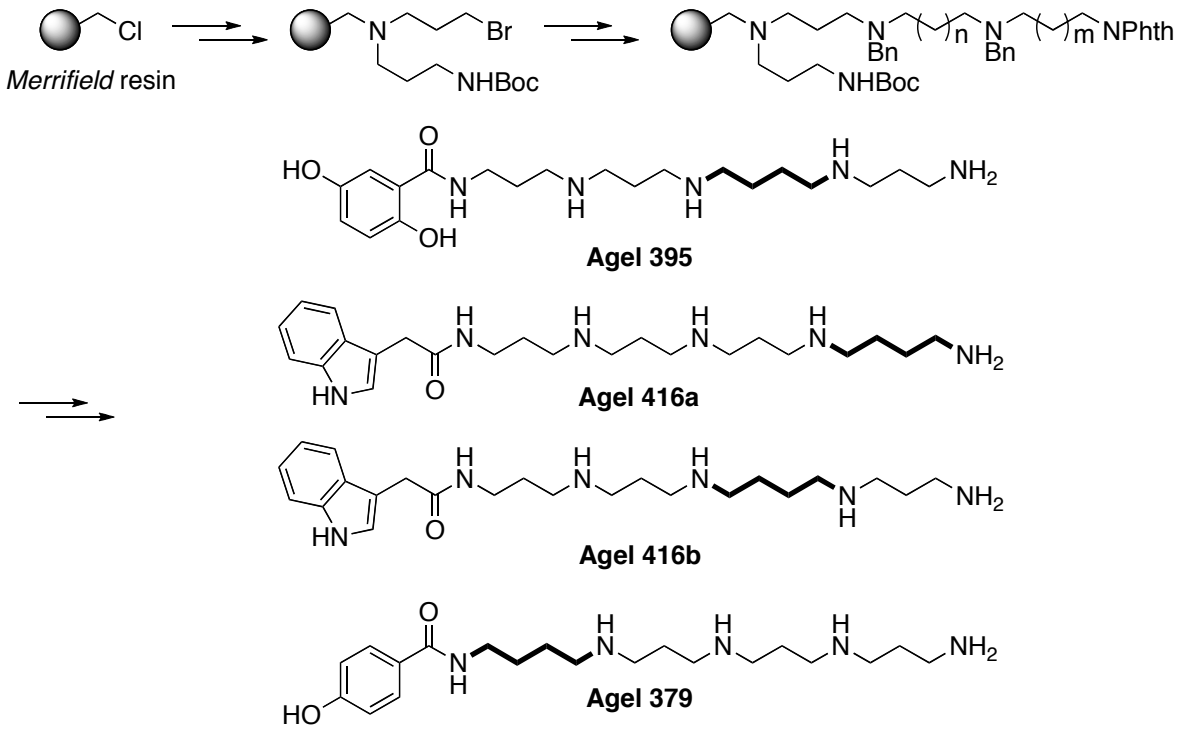
Scheme 6. Combinatorial library of Philanthotoxins by *Strømgaard*.

Hone and *Payne* presented in 2000 a sequential synthesis of **Agel 416** applying the *Fukuyama-Mitsunobu* reaction to the solid-support.[79] The entire synthesis of the spider toxin, except the final deprotection of the Boc protective groups, was realised on solid-phase (Scheme 7). A year later, using the same alkylation conditions as *Hone* and *Payne*, *Strømgaard* prepared the wasp toxin **PhTX-433** and seven analogs of this compound.[80] Up to now, *Andersen*, *Strømgaard* and *Olsen* concentrated on these reaction conditions to improve them and showed, for instance, that the reverse reactivity is also possible.[81-84]



Scheme 7. *Fukuyama-Mitsunobu* reaction in the solid-phase synthesis of **Agel 416**.

In 2001, our group elaborated a new method for the solid-phase synthesis of linear polyamines and polyamine derivatives using the inexpensive *Merrifield* resin.[57,85,86] Starting "from the centre", pentaamine natural products (Scheme 8) but also cyclic tri- and tetraamine derivatives were constructed (Figure 11).



Scheme 8. Linear acyl pentaamine natural products starting from the centre.

Figure 11. Cyclic polyamine derivatives.

4.3. Tools to Follow Reactions

The main disadvantage of working on a solid-phase is the problem that progress of a reaction can hardly be followed directly. For instance, TLC or GC analyses are not possible with solid-bound substrates. Nevertheless, some tools are still available to overcome at least partly this difficulty. Most of them, however, have to be considered as qualitative rather than quantitative hints. The simplest way to follow a reaction is the so-called *Fourier* transformation infrared (FT-IR). The resin is used directly without preliminary treatment. Functionalities with strong absorption are seen but because of the stucture of the polymer itself, aromatic systems cannot be detected. Even if this type of

measurement can give good hints concerning the presence or the absence of functionalities, it is impossible to estimate whether the reaction went to completion or not.

Titration provides a convenient method for quantification of halogens, for instance the *Volhard* titration.[87] After the loading of a starter moiety to the *Merrifield* resin, the yield and the loading capacity (mol g^{-1}) of the resin, which is the most important parameter for the rest of the synthesis, can be determined precisely.

The *Kaiser* test[88] was initially performed to detect the presence of primary amines and concurrently confirmed the removal of a protective group during peptide synthesis.

In principle, NMR spectra of solids, also resin-bound samples, can be recorded but a special equipment would be required. This method is called magic angle spinning NMR. The swollen sample spins at a frequency of 1–2 kHz at the "magic angle" relative to the magnetic field. This technique allows two dimensional NMR experiments to be performed, too.

5. Aims of the Work

In the course of our investigations of spider venom, the decomposition reaction of *N*-hydroxylated compounds was observed in APCI-MS. To the best of our knowledge, the formation of such artifacts has not been reported in literature so far for this class of components. We thus were eager to learn more about this type of in-source reduction to gain better understanding of its prerequisites, and, thus, to obtain a mean to recognise and control it. Consequently, we were interested in extending our solid-phase strategy for the development of a method for the synthesis of sets of *N*-hydroxylated polyamine derivatives. The elaborated pathway will give us access to the preparation of model compounds for the MS studies of the reductive phenomenom but also to reference components for the identification of constituents of venom sample from the spider *Agelenopsis aperta* by HPLC-MS.

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— Chapter 2 —

Development of a Method for the Construction of *N*-Hydroxylated Polyamine Backbones*

Until now, our group has contributed to the preparation of terminally acylated polyamines and cyclic tri- and tetraamine derivatives only, which are not further modified at the internal N-atom.[1-3] A number of natural products, however, are additionally derivatised at the internal N-atoms, like, e.g., the *N*-hydroxylated spider toxins **Agel 448** and **Agel 452** of *Agelenopsis aperta* (Figure. 1).[4,5] In connection with our ongoing studies in structure analysis and synthesis of spider toxins we were interested in extending our solid-phase strategy for the preparation of such *N*-hydroxylated polyamine derivatives, too.

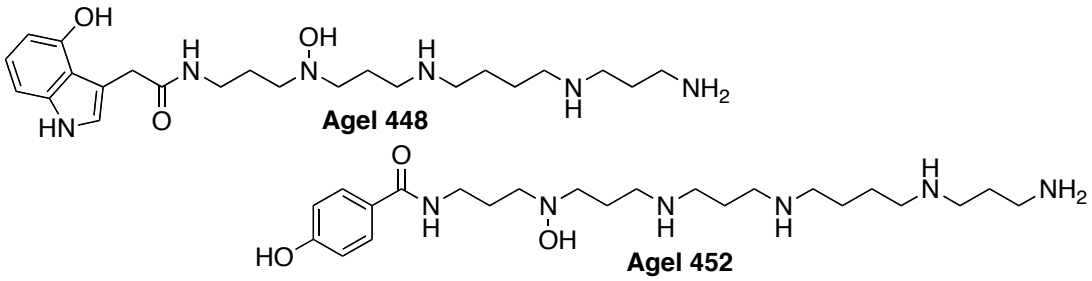


Figure 1. Representatives of *N*-hydroxylated polyamine spider toxins from *Agelenopsis aperta*.

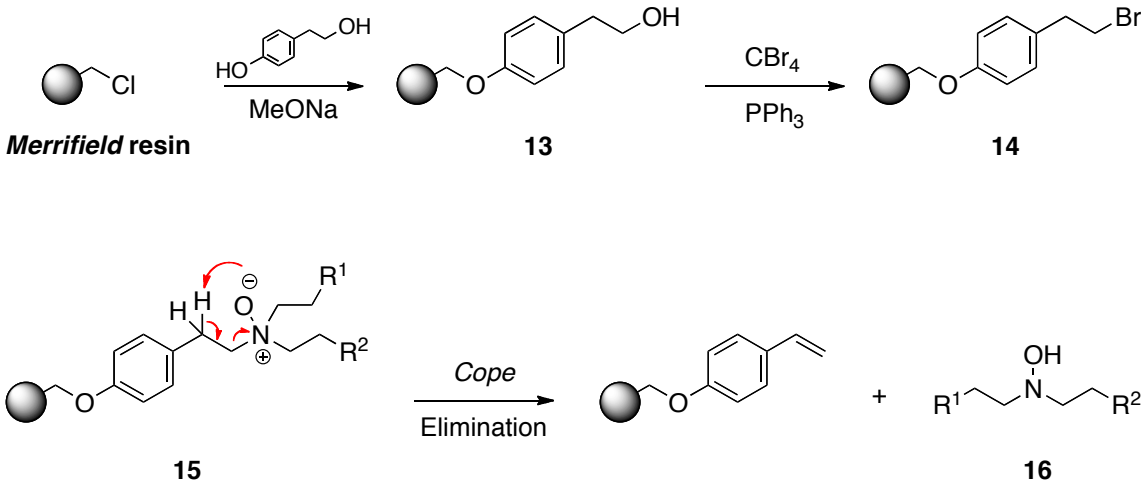
1. Introduction of the Desired *N*-Hydroxy Functionality

The usual method to cleave polyamine derivatives from the *Merrifield* resin is the treatment of the polymer-bound substrates with 1-chloroethyl chloroformate (ACE-Cl) followed by hydrolysis with MeOH. Poor yields obtained with this method for specific polyamine derivatives led us to introduce a

* Part of this chapter is published by Michaël Méret and Stefan Bienz, *Eur. J. Org. Chem.* **2008**, *33*, 5518–5525.

linker in-between the *Merrifield* support and the polyamine in elongation. The appropriate choice of the linker group, however, is crucial to perform a efficient solid-phase synthesis. The attachment point of the linker to the polymer should be chemically inert throughout the synthesis including the cleavage procedure. Its loading should be as quantitative as possible. It should be stable under a rather broad range of reaction conditions and still allow the product to be efficiently cleaved from the solid-support in a very selective manner. With the *Cope* elimination we were confident to have an efficient transformation at hand, which would allow the concurrent introduction of the desired *N*-hydroxy functionality and the cleavage of the final products from the resins. This reaction was already applied for the synthesis of *N*-hydroxylated compounds on solid-phase by *Seo* et al.,[6] however, for simple systems only. The linker introduced contains the phenethyl bromide moiety to be used to attach the amine to the resin (see structure **14**, Scheme 1). The phenethyl group should secure the regioselectivity of the planned *Cope* elimination. Due to the enhanced acidity of benzylic H-atoms, *Cope* elimination of an amine oxide of type **15** should predominantly proceed toward the linker side of the molecule — forming a styrene derivative together with the desired polyamine derivatives **16** — rather than to the side of the polyamine backbone (Scheme 1). To attach the phenethyl bromide linker to the *Merrifield* resin, the latter (200–400 mesh, 2% divinylbenzene, 1.6 mmol g^{-1} loading capacity) was treated analogously to *Seo* et al. for several hours at 50 °C with an excess of 2-(4-hydroxyphenyl)ethanol and MeONa in NMP (1-methyl-2-pyrrolidone). Subsequently, the hydroxyl group of the thus obtained resin **13** was exchanged for a bromide by reaction with PPh_3 and CBr_4 (resin **14**). The transformations were controlled by FT-IR and by *Volhard* titration.[7] Resin **13** showed in IR a broad absorption band at 3350 cm^{-1}, assigned to ν_{OH} of the hydroxyl group, which was not present in the spectrum of the original *Merrifield* resin and which disappeared again for resin **14** (Figure 2). *Volhard* titration of resin **13** showed that the substitution reaction at the *Merrifield* resin with the phenol was complete: no chloride was found, thus, all available substitution sites of the *Merrifield* resin were occupied. Titration of the resin **14** on the other hand,

revealed a halogen content that corresponded to the loading-capacity of the original resin (determined by *Volhard* titration as well).



Scheme 1. The linker / *Cope* elimination.

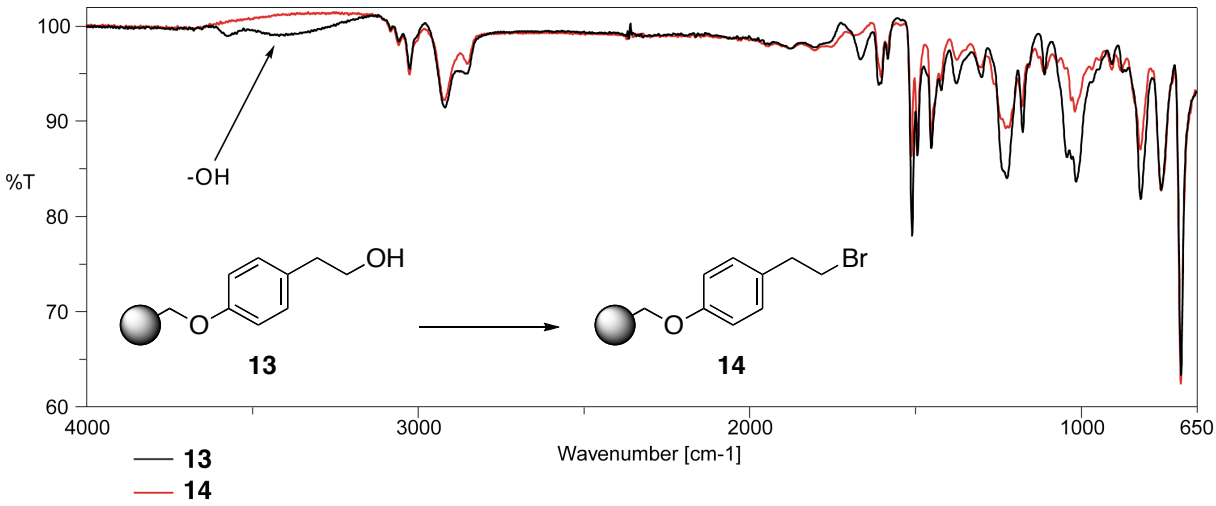


Figure 2. FT-IR overlay of **13** and **14**.

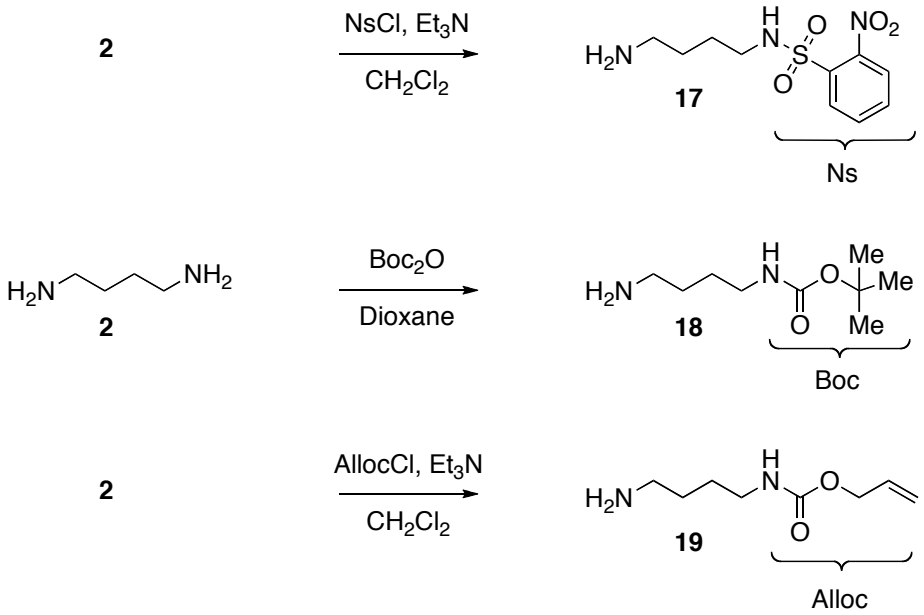
2. Synthesis of Spermidine Derivatives

Since resin **14** and the *Cope* elimination has been used only for the solid-phase synthesis of most simple *N*-hydroxydialkylamines, the new challenge for us was to expand the method to the synthesis of more complex compounds such as *N*-hydroxylated polyamine toxins of **Agel 448** or **Agel 452** type. Solid-phase syntheses of such compounds on resin **14** would not only require

orthogonal protective groups strategies — as syntheses of common non-hydroxylated polyamine derivatives — but also, and particularly, protective groups that are resistant to oxidative conditions.

2.1. Preparation of the Building Blocks

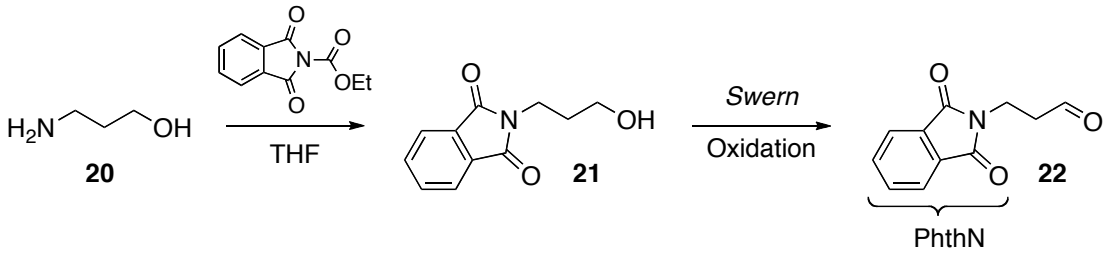
To construct the backbone of derivatives of PA3(OH)4, mono-protected diamines were required, which could be loaded to resin **14** to start the on-resin synthesis (Scheme 2). Three compounds with three different protective groups, chosen with regard to their different behaviour toward cleavage conditions, were prepared. Diamine derivatives **17** and **19** were obtained by reaction of a two fold excess of putrescine (**2**) with 2-nitrophenylsulfonyl chloride (NsCl) and allyl chloroformate (AllocCl), respectively, in presence of Et_3N in CH_2Cl_2.[8,9] Derivative **18** was obtained by treatment of putrescine with Boc anhydride in dioxane.[10]



Scheme 2. Synthesis of the starting building blocks.

As a second type of building block, a protected amino aldehyde, was required to be used for the elongation of the solid-bound diamine derivatives. 3-Phthalimidopropanal (**22**) was chosen as this compound and it was prepared starting with amino alcohol **20** (Scheme 3). In a first step, the amine

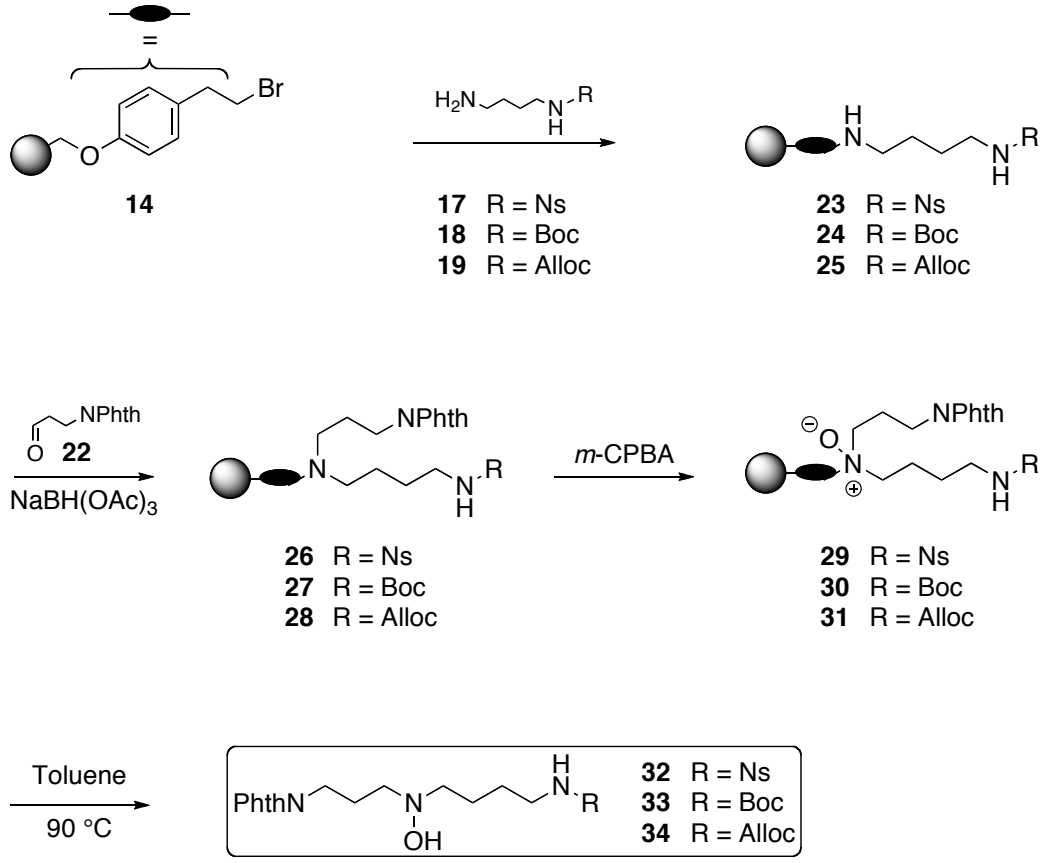
functionality of **20** was protected with the phthaloyl group[11] and subsequently, the alcohol functionality of **21** was converted into an aldehyde group (**22**) by oxidation under *Swern* conditions.[12]



Scheme 3. Synthesis of the elongation building block.

2.2. Solid-Phase Synthesis of the Polyamine Backbone

For the on-resin synthesis of the differently protected polyamine derivatives, resin **14**, was initially loaded with several polyamine starters by nucleophilic substitution. Treatment of resin **14** with an excess of the mono-protected diamines **17–19,** in presence of *N*-diisopropylethylamine (DIEA) in NMP at 50 °C gave rise to resins **23–25** (Scheme 4).



Scheme 4. Solid-support preparation of *N*-hydroxy triamine derivatives.

Volhard titration[7] revealed complete substitution of the halogens. In case of resins **24** and **25** with their Boc- and Alloc-protected amine functionalities, FT-IR showed the characteristic absorption for the carbamate carbonyl group at 1707 cm^{-1} (Figure 3). Concerning the Ns protecting group, FT-IR showed the typical absorptions at 1509 and 1339 cm^{-1} for the nitro group and at 1362 and 1163 cm^{-1} for the sulfonamide.

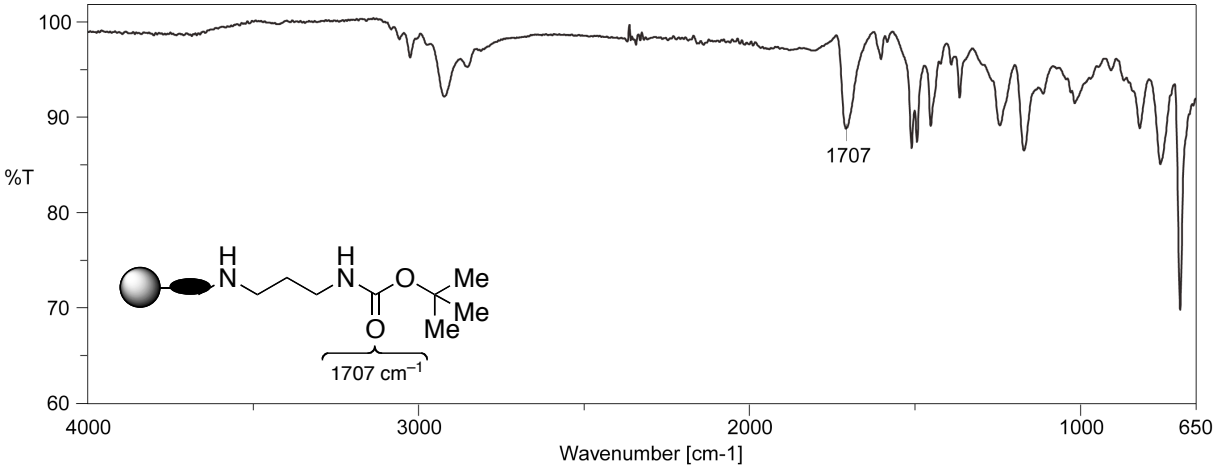


Figure 3. FT-IR of resin **24**.

Elongation of the polyamine backbones was performed by reductive aminations with aldehyde **22**. Treatment of resins **23–25** with this compound for 2 h at 23 °C, followed by reaction with $NaBH(OAc)_3$ in DMF for 2 h at 23 °C, afforded the three resins **26–28**.

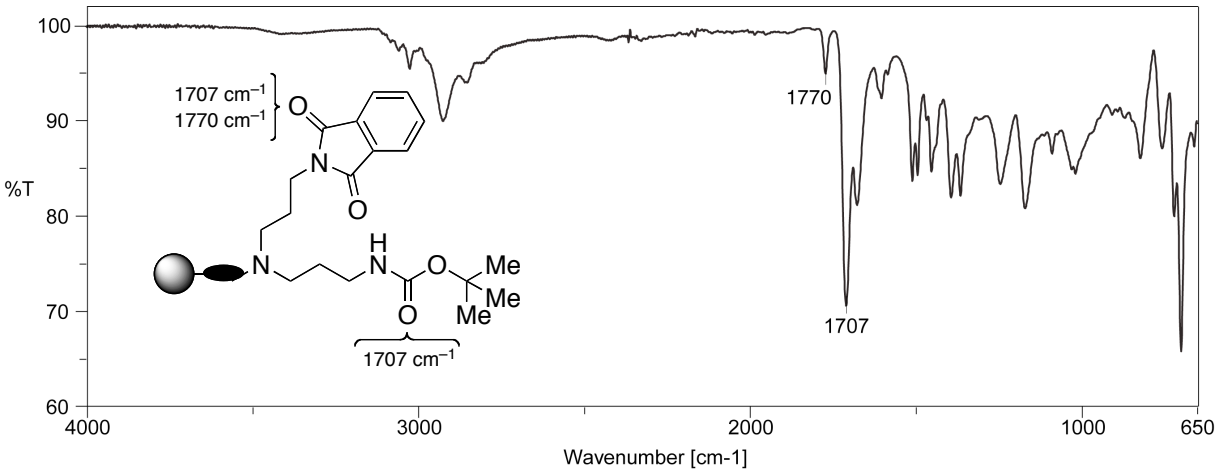


Figure 4. FT-IR of resin **27**.

FT-IR of resins **26–28** showed the new typical absorptions for the carbonyls of the phthalimide groups at 1770 cm^{-1} in addition to the bands at 1707 cm^{-1} for the absorptions of the carbamates (Figure 4) or to the bands at 1509, 1362, 1339 and 1163 cm^{-1} for the absorptions of the Ns group (Figure 5).

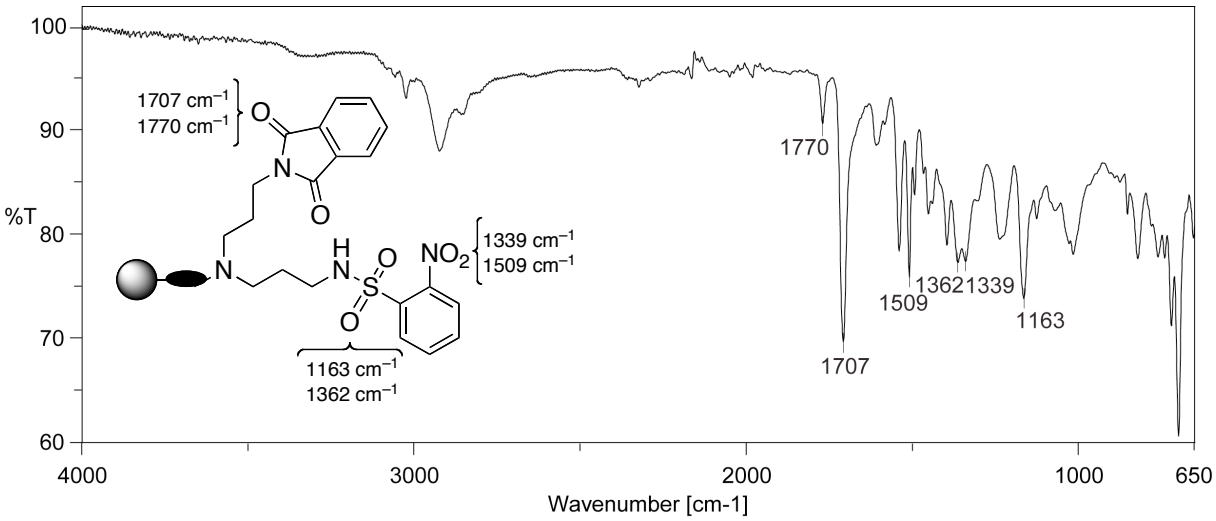


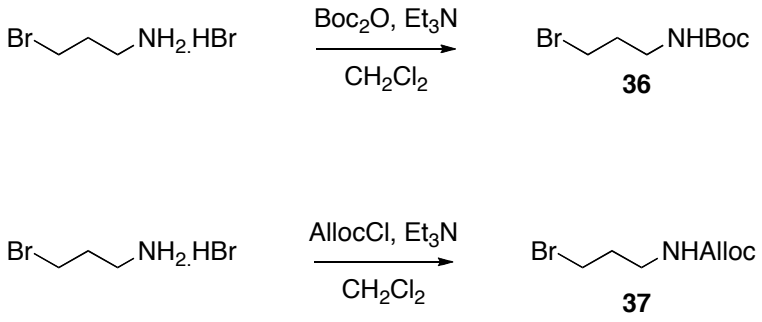
Figure 5. FT-IR of resin **26**.

To obtain the first set of *N*-hydroxylated polyamine derivatives, resins **26–28** were oxidised at 23 °C with *m*-CPBA (3-chloroperbenzoic acid) in CH_2Cl_2. The intermediary amine oxide resins **29–31** were not further characterised but immediately heated in toluene to 90 °C to effect *Cope* elimination and liberation of the desired *N*-hydroxylated spermidine derivatives **32–34**. These products were contaminated with approximately 1% of over-oxidised products — possibly nitrones such as the APCI-MS decomposition products observed with hydroxylamines[13] — as revealed by HPLC-MS of the crude mixtures. Reversed phase HPLC finally furnished the bis-protected *N*-hydroxylated triamines **32–34** in 30–32% overall yields (from resin **14**). Though apparently low, these yields are comparable to those obtained in the solid-phase synthesis of non-hydroxylated polyamine derivatives[1,2] and they are well competitive to the yields that were obtained in syntheses of *N*-hydroxylated polyamine derivatives in solution.[14] The products **32–34** were fully characterised by NMR, ESI-MS and MS/MS, which confirmed the proposed structures as shown in Scheme 4.

3. Synthesis of Spermine Derivatives

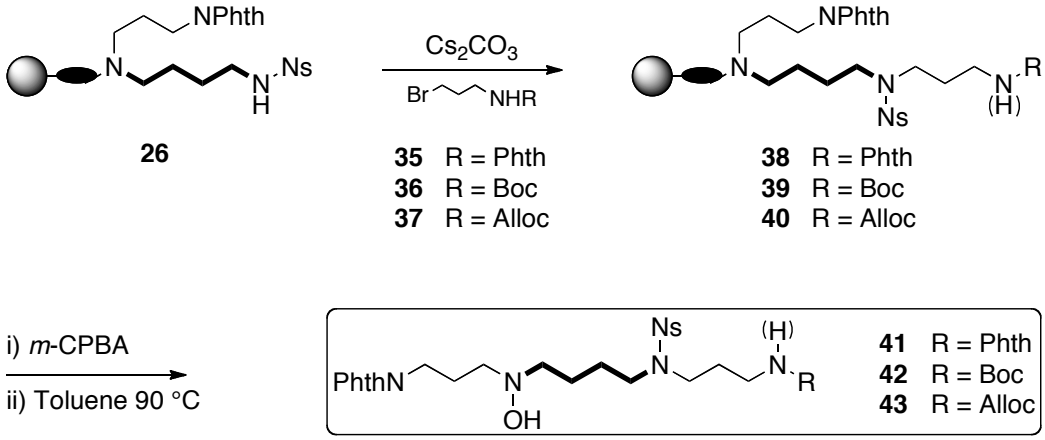
3.1. Backbone 3(OH)43

After the successful synthesis of the serie of diprotected *N*-hydroxylated triamines **32–34,** the synthetic process was further extended to the preparation of a collection of triprotected *N*-hydroxylated tetraamines (Scheme 6). To this purpose, intermediate **26** was alkylated at the NHNs-group with three differently *N*-protected aminobromides, compounds **35–37**. While compounds **35** was commercially available, the derivatives **36** and **37** were synthesised from 3-bromopropylamine hydrobromide[8] as shown in Scheme 5.



Scheme 5. Protected aminobromo building blocks preparation.

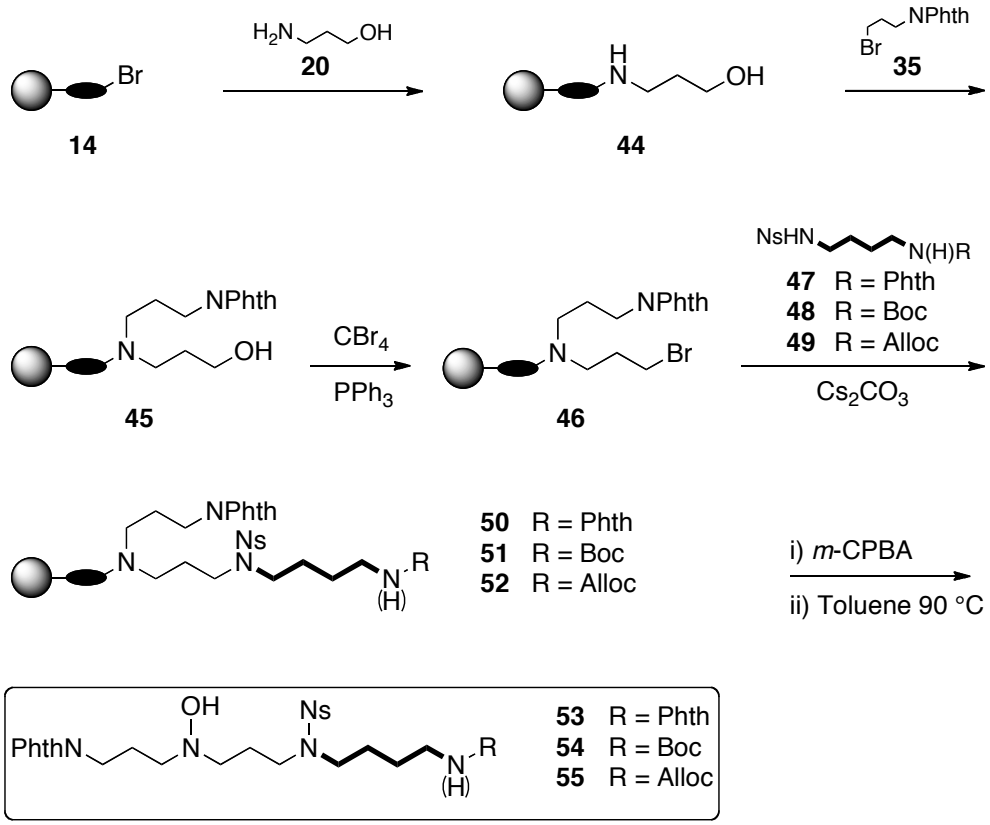
Treatment of resin **26** with compounds **35–37** at 50 °C in presence of Cs_2CO_3 in DMF gave tetraaminic resins **38–40**, which were submitted to the usual oxidation/cleavage conditions to deliver *N*-hydroxyl spermine derivatives **41–43** (28–30% yield from resin **14**).



Scheme 6. Solid-support preparation of 3(OH)43 *N*-hydroxy tetraamine derivatives.

3.2. Backbone 3(OH)34

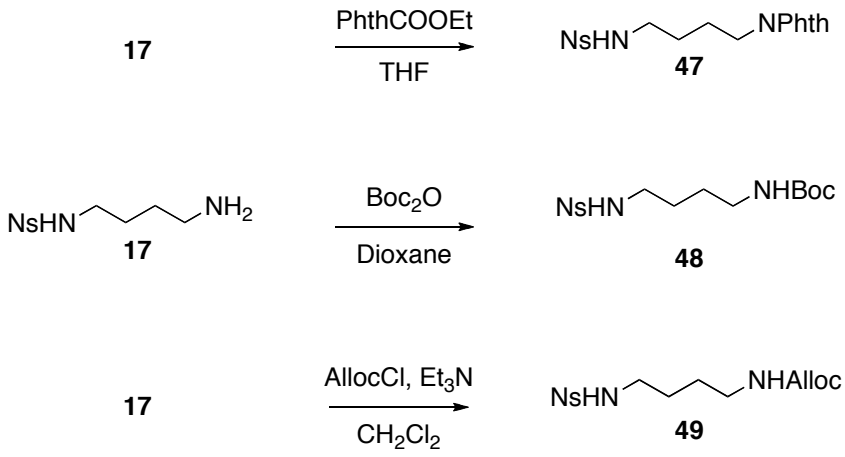
Since spider toxins vary in the succession of the oligomethylene units in-between the several N-atoms,[4] a second set of protected *N*-hydroxy tetraamines with the $N(CH_2)_3N(OH)(CH_2)_3N$ portion in the backbone was prepared (Scheme 7).



Scheme 7. Solid-support preparation of 3(OH)34 *N*-hydroxy tetraamine derivatives.

To show the flexibility of our approach, the construction of the backbone followed a different scheme. Resin **14** was loaded with amino alcohol **20** in presence of DIEA in NMP, affording resin **44**. Alternatively to the reductive amination performed before, the amine of this resin was directly alkylated by treatment with protected aminobromide **35**, in presence of DIEA in NMP at 50° C, leading to resin **45**. Oxidative cleavage revealed that no over-alkylated product was formed in this transformation. The hydroxyl group of resin **45** was then substituted for a bromide by reaction with PPh_3 and CBr_4 in CH_2Cl_2 (resin **46**).[6,15-17] Treatment of the latter resin with nosyl derivatives **47–49**

(obtained from putrescine derivative **17** as shown in Scheme 8) in presence of Cs_2CO_3 in DMF — reversing the reactivities of the resin and the reagents relative to the alkylations of resin **26** with bromides **35–37** — resulted in resins **50–52**, which were oxidatively cleaved as before to furnish the spermine derivatives **53–55** (18–21% yield with respect to resin **14**).



Scheme 8. Preparation of diprotected putrescine building blocks.

The extention of the polyamine backbones was not as trivials as it might appear from the schemes shown above. For the synthesis of the compounds **38–40**, the use of the combination of Cs_2CO_3 with protected aminobromides in the elongation step proved to be superior to a number of alternative conditions. Initially, we employed NaOMe as the base to deprotonate the sulfonamide **26**, but HPLC-MS of the mixtures obtained after treatment of the anions with bromides **35–37**, followed by oxidative cleavage of the product revealed that the alkylation reactions did not proceed to completion (Figure 6): as the major product, the non-alkylated *N*-hydroxyl "starting material", triamine derivative **32**, was obtained. The use of alkyl iodides instead of the bromides was not advantageous, delivering the same triamine derivative **32** as the major product.

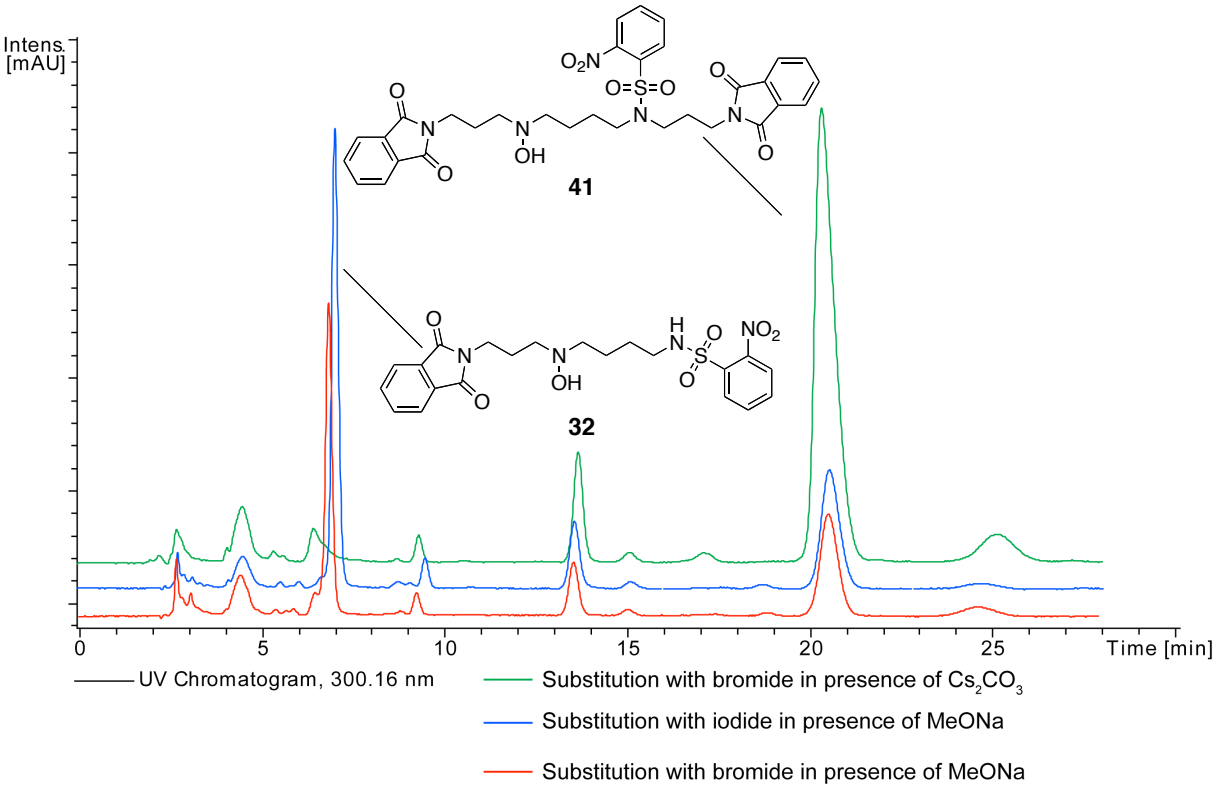
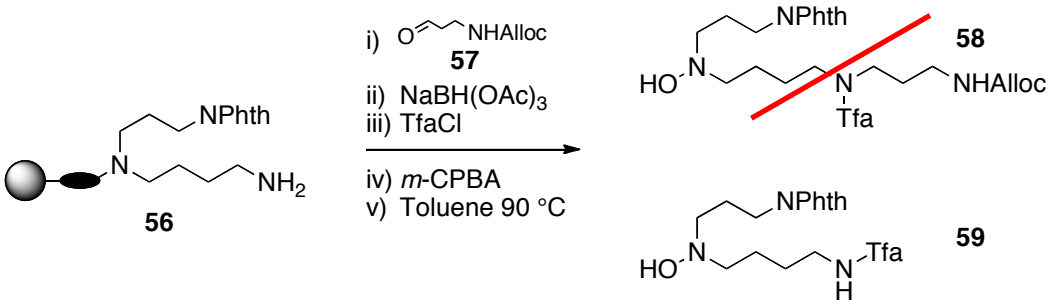


Figure 6. HPLC profiles of the improvement of the substitution step.

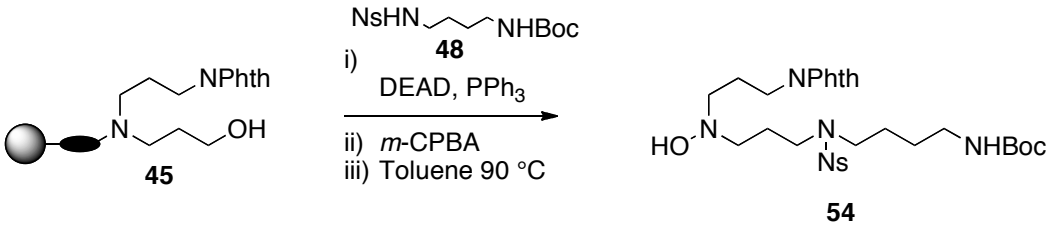
Other synthetic methods to effect elongation of the polyamine backbone were tested, too, but proved not to be better. Our attempts to construct the tetraamine derivatives by successive reductive aminations failed completely (Scheme 9): subsequent treatment of resin **56** with allyl-*N*-(3-oxopropyl)carbamate (**57**) and with $NaBH(OAc)_3$, analogously to the conversions of resins **23–25** into resins **26–28**, furnished a resin showing "negative" *Kaiser* reaction[18] as it was expected for the supposedly formed secondary amine (no more primary amines). After treatment with trifluoroacetyl chloride[19] and the usual oxidative cleavage, however, diprotected *N*-hydroxylated triamine derivative **59** rather than the desired triprotected *N*-hydroxylated tetraamine **58** was obtained. Evidently, and despite the "negative" *Kaiser* test[18] of the intermediary formed resin, the reductive amination was not successful. Two hypotheses were proposed to explain the negative test result for primary amines. Either (1) the imine formed initially in the reductive amination process was not reduced and also not hydrolysed during the subsequent work-up of the reaction or (2) no reaction occurred but complexation of the boron by the

primary amine, and, as a consequence, the primary amine was not detected by the *Kaiser* test[18] in either case.



Scheme 9. Alternative approaches: Successive reductive aminations.

The alternative approach, applying the *Mitsunobu* reaction[20-25] to couple diprotected diamine **48** to the resin through substitution of alcoholic resin **45**, was problematic as well (Scheme 10). In fact, after oxidative cleavage, tetraamine derivative **54** was obtained once in high yield by this procedure, but the method proved to be of low reproducibility in our hands.



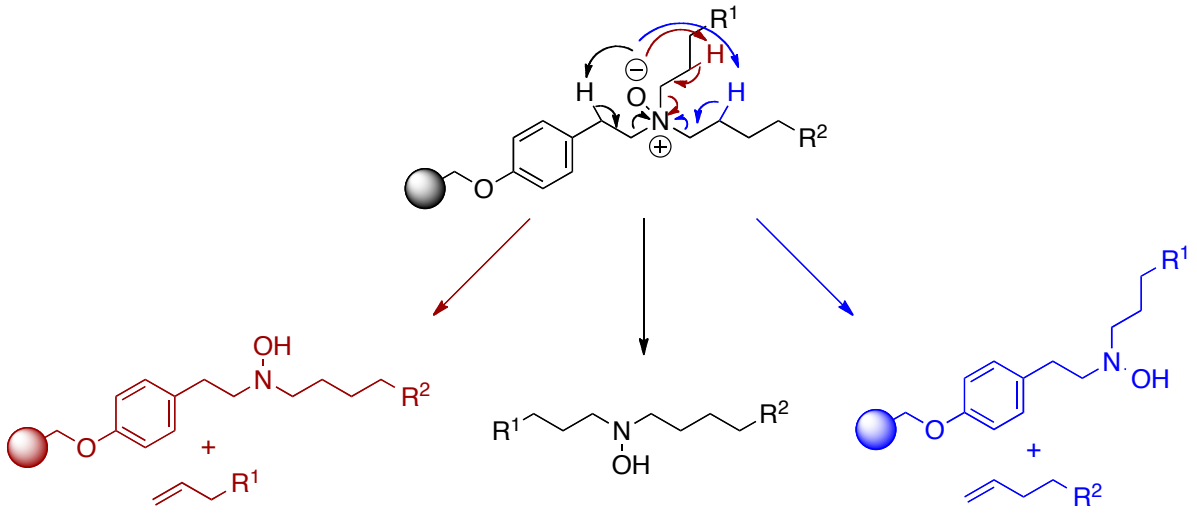
Scheme 10. Alternative approaches: *Mitsunobu* conditions.

4. Toward a New Linker ?·

The obtained yields for the solid-phase synthesis of *N*-hydroxylated tri- and tetraamine derivatives were not high but acceptable. With 30–32% for the triamine and 18–30% for the tetramine derivatives they lie in the range of the yields obtained in solid-phase syntheses of linear non hydroxylated polyamine derivatives and similar to those obtained by "in solution" prepration of *N*-hydroxylated polyamines.

· All experiments on the study of an alternative linker were performed by *Denise Pauli* within her « Diploma Work » in our group.

Nevertheless, the regioselectivity of the final *Cope* elimination was not kown and was regarded as a source for loss of material. The *Cope* elimination was desired to proceed towards the phenethyl moiety of the derivatised resin and not towards the two branches of the polyamine portion. Due to enhanced acidity of the benzylic H-atoms, the reaction in fact leads predominantly to the desired product (Scheme 11).



Scheme 11. Regioselectivy of the *Cope* elimination.

The extent of undesired *Cope* elimination and the potential to improve the side selectivity of the reaction remained in the dark. To investigate the regioselectivity of the *Cope* elimination, and to test modified linker moieties as alternative auxiliaries, the three arylethyl bromide **60**–**62** (Figure 7) were prepared. Compound **60** mimics the actual linker, the benzyloxy group simulating the link to the solid-support. The non-substituted phenyl compound **61** has the function to give us a hint on the effect of the « link to the polymer » on the selectivity of the elimination, and, finally, compound **62** represents the model of a new linker that possesses the benzyloxy group (linker-unit) and additionally an other nitro group to enhance the acidity of the benzylic H-atoms. As a polyamine model compound, spermidine derivative **63**, protected at both terminal amines with a phthaloyl group was chosen.

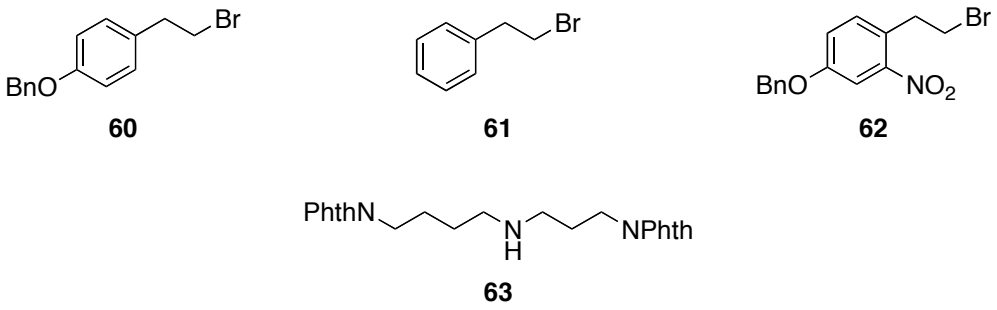
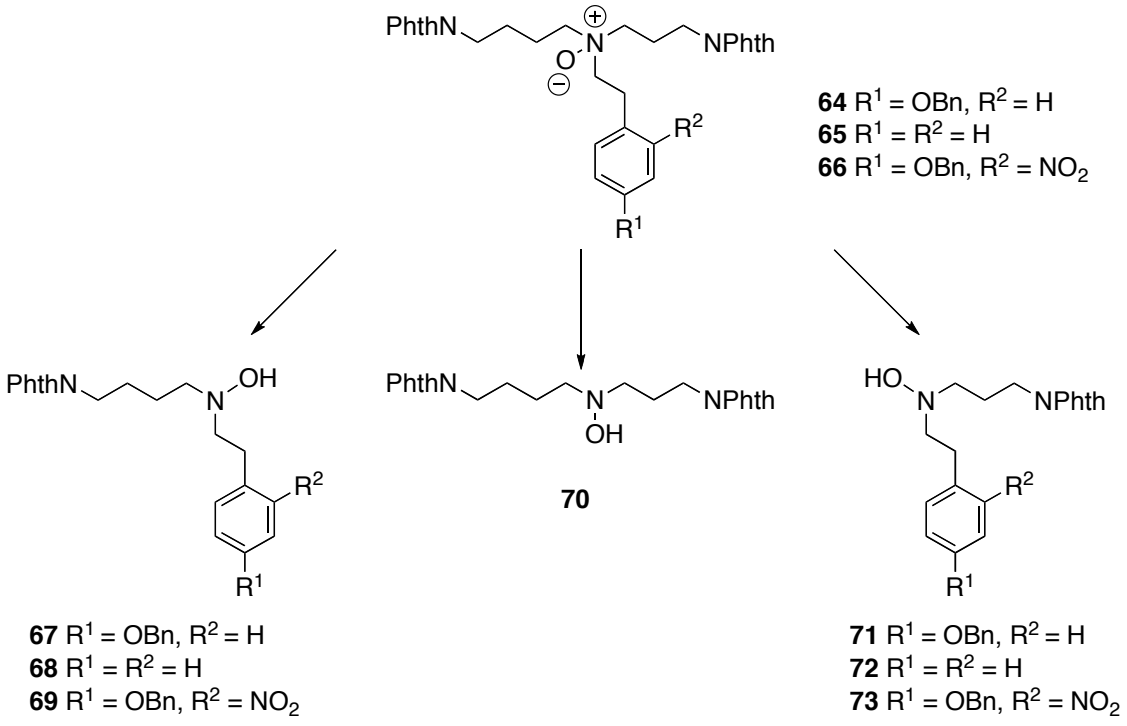


Figure 7. The three model linkers and the model spermidine derivative.

The polyamine model **63** was thus attached to the three different linker models by nucleophilic substitution (surprisingly yields were not as high as expected), and the obtained tertiary amines were oxidised with *m*-CPBA to deliver the amine oxide derivatives **64–66**. These substrates were submitted to the usual cleavage conditions that were applied in the solid-phase chemistry (toluene, 90 °C, 2 h), which led to the *Cope* elimination and to the formation of the expected major product **70** together with minor amounts of compounds of type **67–69** and **71–73** (Scheme 12).



Scheme 12. Products of the *Cope* elimination.

The ratios of the different elimination products were estimated by HPLC-MS through integration of the UV absorbance and are shown in Table 1.

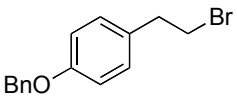
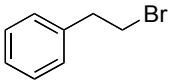
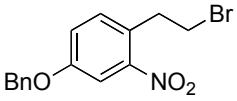
Linker	Product	Amount (%)
 **60**	**70**	**81**
	67	14
	71	5
 **61**	**70**	**90**
	68	8
	72	2
 **62**	**70**	**97**
	69	2
	73	1

Table 1. Ratios of the *Cope* elimination.

The fraction of the desired product **70** for the three reactions demonstrates the very different behaviour of the three amine oxides regarding the regioselectivity of the *Cope* elimination. The non-substituted model furnished 90% of desired *N*-hydroxy spermidine derivative **70** whereas the model simulating the solid-phase bound linker afforded 81% of **70** only. Introduction of the nitro group, on the other hand, increased considerably the amount of the desired product **70**, giving almost a quantitative yield. These results show that in the cleaving step approximately 20% of desired hydroxylamines are lost because of the linkage to the resin through a *para* alkoxy function, deactivates the substrat for the desired reaction. The results show also, and especially, that this effect can be overcompensated by the introduction of a nitro group in *ortho* position of the arylethyl moiety.

The results of this in-solution investigation did not find entry in synthetic application yet. Some synthetic problems with regard to the preparation of the linker unit and the loading reaction have to be solved first. The respective investigation has started just recently as well as the study of the new linker as

a framework to be used for the solid-phase preparation of *N*-methyl polyamines *via Hofmann* elimination.

5. Conclusions

In conclusion, we succeded to synthesise diprotected *N*-hydroxy spermidines and triprotected *N*-hydroxylated spermines and spermine analogues on solid-support, and we have demonstrated that Ns, Boc, Alloc and Phth protecting groups are compatible with the oxidative cleavage procedure. We have shown that the approach for the synthesis of the polyamine backbones on the solid support is flexible, allowing the construction of the resin-bound polyamine portion by reductive aminations, direct alkylations of amines and sulfonamides as well as substitutions of halogenides. We thus have laid the basis for the solid-phase synthesis of any *N*-hydroxylated polyamine derivatives. The synthesised *N*-hydroxypolyamine derivatives will be used as precursors for synthetic polyamine natural products and as reference compounds for the study and identification of constituents of venom samples of the spider *Agelenopsis aperta*, applying HPLC-MS.

6. EXPERIMENTAL SECTION

Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. TLC: silica gel 60 F_{254} on aluminium sheets (*Merck*). Chromatography: *Merck* silica gel 60 (40–63 μm). Resin used: *Merrifield* polymer 200–400 mesh, 2% divinylbenzene, loading ~2.1 mmol Cl g^{-1} resin from *Fluka*. For the solid-phase reactions an *Advanced ChemTech PLS 6 Organic synthesiser* was used. IR spectra were recorded on a *Perkin–Elmer* 1600 Series FT-IR spectrophotometer and for the final products, an *OMNILAB* FT/IR 4100 spectrophotometer. *N*-Hydroxypolyamine derivatives were purified by preparative HPLC; chromatograms were recorded with *Dynamax* solvent delivery system model SD-300 coupled with a *Dynamax* absorbance detector model UV-1; column used: *Kromasil* KR100-10C18. Routine ^{1}H-NMR spectra in $CDCl_3$ were measured with a *Bruker* AC-300 (300 MHz); δ rel. to $CHCl_3$ (δ 7.26 ppm). Routine ^{13}C-NMR spectra in $CDCl_3$ were measured with a *Bruker* AC-300 (75.5 MHz); δ rel. to $CDCl_3$ (δ 77.0 ppm). Final products ^{1}H-NMR spectra in $CDCl_3$ were measured with a *Bruker* AV-600 (600 MHz); δ rel. to $CHCl_3$ (δ 7.26 ppm). Final products ^{13}C-NMR spectra in $CDCl_3$ were measured with a *Bruker* AV-600 (150 MHz); δ rel. to $CDCl_3$ (δ 77.0 ppm); multiplicities from DEPT-135 and DEPT-90 experiments; the assignments of the carbon resonances followed from HSQC experiments. ESI-MS was performed on a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH*, Bremen, Germany), equipped with a combined *Hewlett-Packard* Atmospheric Pressure Ion (API) source (*Hewlett–Packard Co.*, Palo Alto, CA, USA). HR-MS: High resolution (≥ 10'000 FWHM) electrospray ionization mass spectrometry was performed on a *Finnigan* MAT 900 (*Thermo Finnigan*, San Jose, CA; USA) double-focusing magnetic sector mass spectrometer. 10 spectra were acquired. A mass accuracy ≤ 2 ppm was obtained in the peak matching acquisition mode by using a solution containing 2 μL PEG200, 2 μL PPG450, and 1.5 mg NaOAc (all obtained from Sigma-Aldrich, CH-Buchs) dissolved in 100 mL MeOH (HPLC Supra grade, *Scharlau*, E-Barcelona) as internal standard. Proof of structure and purity of the

final polyamine derivatives was provided by NMR spectra and MS/MS. Elemental analyses were not appropriate for the polyamine derivatives since the compounds arose as waxy or glassy solids only, from which the last solvent residues can hardly be removed.

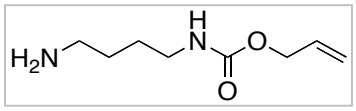
6.1. Preparation of the Building Blocks

***N*-(2-Nitrophenylsulfonyl)-1,4-diaminobutane (17).** To a solution of 1,4-diaminobutane (40.0 mL, 399.3 mmol) and Et_3N (27.96 mL, 200.8 mmol) in dry CH_2Cl_2 (150 mL) was added dropwise a solution of 2-nitrophenylsulfonyl chloride (44.51 g, 200.8 mmol) in dry CH_2Cl_2 (150 mL) at 0 °C. The mixture was stirred at 23 °C for 3 h. The precipitate was filtered off and washed with CH_2Cl_2. Water was added and the organic phase was extracted, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow solid (25.79 g, 94.4 mmol, 47% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 8.28–8.25 (m, 1 H, arom. H *o* to NO_2), 7.97–7.93 (m, 1 H, arom. H *o* to SO_2), 7.89–7.84 (m, 2 H, arom. H *p* to NO_2 and arom. H *p* to SO_2), 3.23 (t, $^3J_{H,H}$ = 6.5 Hz, 2 H, NsNHCH_2), 2.84 (t, $^3J_{H,H}$ = 6.5 Hz, 2 H, NH_2CH_2), 1.76 (quint, $^3J_{H,H}$ = 6.5 Hz, 2 H, NsNHCH$_2$CH_2), 1.62 (quint, $^3J_{H,H}$ = 6.5 Hz, 2 H, $NH_2CH_2CH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 133.9 (d, arom. CH *p* to NO_2), 133.2 (s, arom. CSO_2), 132.4 (d, arom. CH *p* to SO_2), 130.9 (d, arom. CH *o* to NO_2), 124.9 (d, arom. CH *o* to SO_2), 43.5, 41.2, 30.4, 27.7 (4 t, 4 CH_2) ppm. ESI-MS *m*/*z* = 274.1 (100, [*M*+H]$^+$).

***tert*-Butyl *N*-(4-Aminobutyl)carbamate (18).** To a solution of 1,4-diaminobutane (12.0 mL, 119.8 mmol) in dioxane (100 mL) under an Ar atmosphere, di-*tert*-butyldicarbonate (24.77 g, 113.5 mmol) dissolved in dioxane (70 mL) was added dropwised at 0 °C. The reaction was stirred overnight at 23 °C then refluxed (120 °C) for 4 h. The mixture was filtered and the filtrat was concentrated *in vacuo* to give a yellow oil (20.30 g, 107.8 mmol, 95% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 3.43–3.07 (m, 2 H, BocNHCH_2), 2.69 (t,

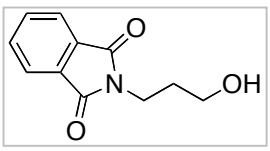
$^{3}J_{H,H}$ = 6.6 Hz, 2 H, NH_2CH_2), 1.52–1.44 (m, 4 H, $CH_2CH_2CH_2CH_2$), 1.42 (s, 9 H, Me_3C) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 155.9 (s, CO), 78.8 (s, Me_3C), 41.8, 30.7 (2 t, 2 CH_2), 28.3 (q, Me_3C), 27.3 (t, CH_2) ppm. ESI-MS m/z = 189.1 (100, $[M+H]^+$), 133.1 (18, $[M–56+H]^+$).

Allyl *N*-(4-Aminobutyl)carbamate (19). To a solution of 1,4-diaminobutane



(40.0 mL, 399.3 mmol) and Et_3N (27.96 mL, 200.8 mmol) in dry CH_2Cl_2 (150 mL) was added dropwise a solution of allyl chloroformate (16.94 mL, 200.8 mmol) in dry CH_2Cl_2 (150 mL) at 0 °C. The mixture was stirred at 23 °C for 12 h. The precipitate was filtered and washed with CH_2Cl_2. Water was added and the organic phase was extracted, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow oil (18.33 g, 106.4 mmol, 53% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 6.06–5.66 (m, 1 H, CH_2=C*H*), 5.29 (dd, $^{3}J_{H,H\text{-}trans}$ = 17.2, $^{2}J_{H,H\text{-}gem}$ = 1.1 Hz, 1 H, CH_2=CH), 5.19 (d, $^{3}J_{H,H\text{-}cis}$ = 9.3 Hz, 1 H, CH_2=CH), 4.55 (br. s, 2 H, OCH_2), 3.17 (br. s, 2 H, AllocNHCH_2), 2.80–2.63 (m, 2 H, NH_2CH_2), 1.72–1.39 (m, 4 H, $CH_2CH_2CH_2CH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 156.2 (s, CO of Alloc), 132.9 (d, CH_2=CH), 117.0 (t, CH_2=CH), 64.9 (t, CH_2=$CHCH_2$), 41.4, 40.5, 30.4, 27.1 (4 t, 4 CH_2) ppm. ESI-MS m/z = 173.1 (100, $[M+H]^+$).

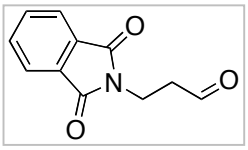
3-Phthalimidopropanol (21). To a solution of 3-aminopropan-1-ol (22.47 mL,



295.6 mmol) in THF (100 mL), *N*-ethoxycarbonylphthalimide (64.79 g, 295.6 mol) was added. The mixture was stirred at 23 °C for 12 h under Ar atmosphere. After completion of the reaction, the solvent was evaporated to give a colourless oil. Purification by flash chromatography (CH_2Cl_2/MeOH 9:1) gave a colourless solid (46.10 g, 224.6 mmol, 76% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.88–7.82 (m, 2 H, 2 arom. CCH of Phth), 7.75–7.69 (m, 2 H, 2 arom. CCHC*H* of Phth), 3.86 (t, $^{3}J_{H,H}$ = 6.4 Hz, 2 H, PhthNCH_2), 3.62 (m, $^{3}J_{H,H}$ = 5.7 Hz, 2 H, HOCH_2), 1.88 (tt, $^{3}J_{H,H}$ = 6.4, $^{3}J_{H,H}$ = 5.7 Hz, 2 H, $CH_2CH_2CH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 168.7 (s, 2 CO of Phth), 133.9 (d, 2 arom.

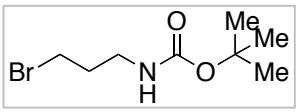
CCHCH of Phth), 131.7 (s, 2 arom. C of Phth), 123.1 (d, 2 arom. CCH of Phth), 58.8, 33.9, 31.1 (3 t, 3 CH_2) ppm. ESI-MS *m*/*z* = 447.2 (36), 228.1 (100, [*M*+Na]$^+$).

3-Phthalimidopropanal (22). A solution of DMSO (19.76 mL, 278.2 mmol) in



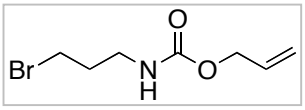
CH_2Cl_2 (50 mL) was added dropwised over 60 min to a stirred solution of oxalyl chloride (11.77 mL, 133.9 mol) in CH_2Cl_2 (30 mL) at –78 °C. After 5 min stirring, 3-phthalimidopropanol (**21**, 14.28 g, 69.6 mmol) in CH_2Cl_2 was added dropwised over 30 min at –78 °C. After 40 min stirring, Et_3N (58.10 mL, 417.4 mmol) was added dropwised over 15 min. the resulting mixture was warmed up to 0 °C and stirred for 1 h. Water was added to quench the reaction. The organic layer was separated, washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo* to give a colourless solid (11.03 g, 54.3 mmol, 78% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 9.80 (t, $^3J_{H,H}$ = 1.3 Hz, 1 H, CHO), 7.86–7.79 (m, 2 H, 2 arom. CCH of Phth), 7.73–7.67 (m, 2 H, 2 arom. CCHC*H* of Phth), 4.02 (t, $^3J_{H,H}$ = 6.9 Hz, 2 H, PhthNCH_2), 2.86 (dt, $^3J_{H,H}$ = 6.9, $^3J_{H,H}$ = 1.3 Hz, 2 H, CHOCH_2) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 199.3 (d, CHO), 167.9 (s, 2 CO of Phth), 133.9 (d, 2 arom. CH), 131.8 (s, 2 arom. C), 123.2 (d, 2 arom. CH), 42.2, 31.5 (2 t, 2 CH_2) ppm. ESI-MS *m*/*z* = 228.0 (100, [*M*+Na]$^+$).

***tert*-Butyl *N*-(3-Bromopropyl)carbamate (36).** 3-Bromopropylamine hydro-



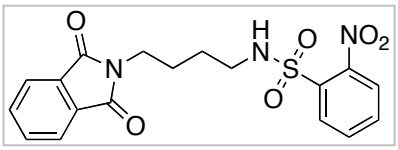
bromide (2.06 g, 9.4 mmol) was suspended in CH_2Cl_2. Et_3N (1.31 mL, 28.3 mmol) was added. The suspension was stirred for 30 min at 23 °C. The solution was cooled down to 0 °C and di-*tert*-butyldicarbonate (2.05 g, 9.4 mmol) was added portionwise. The suspension was stirred for 12 h at 23 °C. Water was added and the organic phase was extracted, dried with $MgSO_4$ and concentrated *in vacuo* to give a slightly yellow oil (1.34 g, 5.6 mmol, 60% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 3.44 (t, $^3J_{H,H}$ = 6.5 Hz, 2 H, $BrCH_2$), 3.30–3.24 (m, 2 H, BocNHCH_2), 2.05 (quint, $^3J_{H,H}$ = 6.5 Hz, 2 H, $CH_2CH_2CH_2$), 1.44 (s, 9 H, Me_3C) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 155.9 (s, CO), 79.3 (s, Me_3C), 38.9 (t, $BrCH_2$), 32.6 (t, CH_2NH), 30.7 (t, $CH_2CH_2CH_2$), 28.3 (q, Me_3C) ppm. ESI-MS *m*/*z* = 281.2 (33), 260.0 (100, [*M*+Na]$^+$), 228.0 (38).

Allyl *N*-(3-Bromopropyl)carbamate (37). 3-Bromopropylamine hydrobromide



(20.65 g, 94.3 mmol) was suspended in CH_2Cl_2. Et_3N (13.12 mL, 94.3 mmol) was added. the suspension was stirred for 30 min at 23 °C. The solution was cooled down to 0 °C and allyl chloroformate (10.00 mL, 94.1 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The suspension was stirred for 12 h at 23 °C. The mixture was filtered, the precipitate washed with CH_2Cl_2. Water was added and the organic phase was extracted, washed with 1N HCl (150 mL), brine, dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow oil (14.21 g, 63.99 mmol, 68% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 5.92 (ddt, $^3J_{H,H\text{-}trans}$ = 17.2, $^3J_{H,H\text{-}cis}$ = 10.5, $^3J_{H,H\text{-}vic}$ = 5.2 Hz, 1 H, CH_2=C*H*), 5.30 (dd, $^3J_{H,H\text{-}trans}$ = 17.2, $^2J_{H,H\text{-}gem}$ = 1.2 Hz, 1 H, CH_2=CH), 5.22 (dd, $^3J_{H,H\text{-}cis}$ = 10.5, $^2J_{H,H\text{-}gem}$ = 1.2 Hz, 1 H, CH_2=CH), 4.57 (d, $^3J_{H,H\text{-}vic}$ = 5.2 Hz, 2 H, OCH_2), 3.45 (*t*, $^3J_{H,H}$ = 6.5 Hz, 2 H, $BrCH_2$), 3.37–3.30 (m, 2 H, AllocNHCH_2), 2.08 (quint, 2 H, $^3J_{H,H}$ = 6.5 Hz, $CH_2CH_2CH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 156.3 (s, CO), 132.7 (d, CH_2=CH), 117.5 (t, CH_2= CH), 65.4 (t, OCH_2), 39.2, 32.4, 30.6 (3 t, 3 CH_2). ESI-MS *m*/*z* = 244.0 (100, [*M*+Na]$^+$).

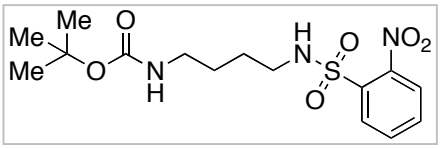
***N*-[4-(2-Nitrophenylsulfonamido)butyl]phthalimide (47).** *N*-Ethoxycarbonyl-



phthalimide (0.80 g, 3.7 mmol) was added portionwise to a solution of *N*-(2-nitrophenylsulfonyl)-1,4-diaminobutane (**17**, 1.00 g, 3.7 mmol) in THF (15 mL) at 0 °C. The solution was stirred for 12 h at 23 °C under Ar atmosphere. Solvent of the reaction was evaporated. Water was added to the residue and the organic phase was extrated with CH_2Cl_2, dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. Purification by flash chromatography (CH_2Cl_2/MeOH 9:1) gave a colourless solid (0.73 g, 1.81 mmol, 49% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 8.15–8.12 (m, 1 H, arom. H *o* to NO_2), 7.87–7.81 (m, 3 H, arom. H *o* to SO_2, 2 arom. CCH of Phth), 7.76–7.69 (m, 4 H, arom. H *p* to NO_2, arom. H *p* to SO_2 and 2 arom. CCHC*H* of Phth), 5.36 (t, $^3J_{H,H}$ = 6.1 Hz, 1 H, NH), 3.66 (t, $^3J_{H,H}$ = 6.9 Hz, 2 H, PhthNCH_2), 3.16 (ap. td, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 6.4 Hz, 2H, NsNHCH_2), 1.77–1.67 (m, 2 H, PhthNCH_2CH_2),

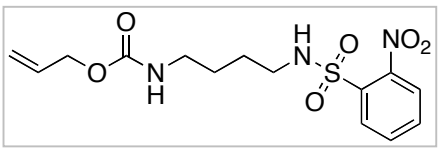
1.62–1.53 (m, 2 H, NsNHCH$_2$C*H*$_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 166.5 (s, 2 CO), 132.2 (d, 2 arom. CCHCH of Phth), 131.7 (d, arom. CH *p* to NO_2), 131.0 (s, arom. CSO_2), 130.2 (d, arom. CH *p* to SO_2), 129.3 (d, arom. CH *o* to NO_2), 123.6 (d, arom. CH *o* to SO_2), 121.5 (s, 2 arom. C of Phth), 125.5 (d, arom. CH *o* to SO_2), 121.5 (d, 2 arom. CCH of Phth), 41.5 (t, NsNHCH$_2$), 35.3 (t, PhthNCH$_2$), 25.1, 23.8 (2 t, 2 CH_2) ppm. ESI-MS *m*/*z* = 481.0 (12), 442.0 (8, [*M*+K]$^+$), 426.1 (100, [*M*+Na]$^+$), 404.1 (5, [*M*+H]$^+$), 368.1 (9).

***tert*-Butyl *N*-[4-(2-Nitrophenylsulfonamido)butyl]carbamate (48).** A solution



of di-*tert*-butyldicarbonate (19.82 g, 90.8 mmol) in dioxane (50 mL) was added dropwise to a solution of *N*-(2-nitrophenylsulfonyl)-1,4-diaminobutane (**17**, 24.82 g, 90.8 mmol) in a 1:1 mixture of DMF/dioxane (50 mL). The solution was stirred for 12 h at 23 °C. The solvents of the reaction were evaporated. Water was added to the residue and the organic phase was extrated with CH_2Cl_2, dried with $MgSO_4$ and concentrated *in vacuo* to give **48** as an orange solid (33.57 g, 89.9 mmol, 99% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 8.13–8.11 (m, 1 H, 1 arom.), 7.85–7.82 (m, 1 H, 1 arom.), 7.74–7.71 (m, 2 H, 2 arom.), 3.11–3.05 (m, 4 H, 2 NHC*H*$_2$), 1.56–1.49 (m, 4 H, CH_2CH_2-CH_2CH_2), 1.41 (s, Me_3C) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 156.0 (s, CO), 133.7 (d, arom. CH *p* to NO_2), 133.6 (s, arom. CSO_2), 132.8 (d, arom. CH *p* to SO_2), 131.1 (d, arom. CH *o* to NO_2), 125.4 (d, arom. CH *o* to SO_2), 43.4, 28.4, 27.2, 26.9 (4 t, 4 CH_2) ppm. ESI-MS *m*/*z* = 372.1 (100, [*M*–H]$^-$).

Allyl *N*-[4-(2-Nitrophenylsulfonamido)butyl]carbamate (49). A solution of

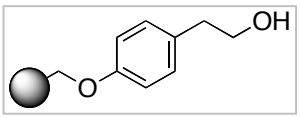


allyl chloroformate (1.55 mL, 14.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of *N*-(2-nitrophenylsulfonyl)-1,4-diaminobutane (**17**) (3.99 g, 14.6 mmol) and DIEA (2.75 mL, 16.1 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After completion of the addition, the solution was warmed up to 23 °C and stirred for 12 h. Water was added and the organic phase was extracted, dried with $MgSO_4$ and concentrated *in vacuo* to give **49** as a yellow oil which cristallized (5.16 g, 14.4 mmol, 99% yield). ^{1}H-NMR (300 MHz,

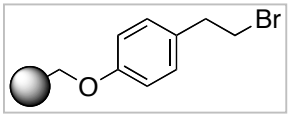
$CDCl_3$, 298 K) δ = 8.15–8.12 (m, 1 H, arom. H *o* to NO_2), 7.89–7.86 (m,1 H, arom. H *o* to SO_2), 7.78–7.73 (m, 2 H, arom. H *p* to NO_2 and arom. H *p* to SO_2), 5.90 (ddt, $^3J_{H,H\text{-}trans}$ = 17.3, $^3J_{H,H\text{-}cis}$ = 10.7, $^3J_{H,H\text{-}vic}$ = 5.3 Hz, 1 H, CH_2=C*H*), 5.30 (dd, $^3J_{H,H\text{-}trans}$ = 17.3, $^2J_{H,H\text{-}gem}$ = 1.5 Hz, 1 H, CH_2=CH), 5.21 (d, $^3J_{H,H\text{-}cis}$ = 10.7 Hz, 1 H, CH_2=CH), 4.54 (d, $^3J_{H,H}$ = 5.3 Hz, 2 H, OCH_2), 3.20–3.09 (m, 4 H, AllocNHCH_2, NsNHCH_2), 1.60–1.54 (m, 4 H, $CH_2CH_2CH_2CH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 278 K) δ = 154.9 (s, CO of Alloc), 146.7 (s, arom. CNO_2), 132.3 (s, arom. CSO_2), 132.0 (d, CH_2=CH), 131.5 (d, arom. CH *p* to NO_2), 131.3 (d, arom. CH *p* to SO_2), 129.6 (d, arom. CH *o* to NO_2), 123.8 (d, arom. CH *o* to SO_2), 116.2 (t, CH_2=CH), 64.1 (t, CH_2=CHCH_2), 51.6, 41.9, 25.6, 25.4 (4 t, 4 CH_2) ppm. ESI-MS *m*/*z* = 396.1 (12, $[M+K]^+$), 380.1 (100, $[M+Na]^+$).

6.2. Synthesis of the Modified Merrifield *Resin*

Attachment of the Phenethyl Alcohol Linker (13). MeONa (0.81 g, 15.0 mmol) was added to a solution of 2-(4-hydroxyphenyl)ethanol (2.07 g, 15.0 mmol) in NMP (60 mL) at 0 °C. The solution was warmed up to 23 °C, mechanically stirred for 2 h, and the *Merrifield* resin (3.13 g, 5.0 mmol, loading capacity determined by *Volhard* titration:[7] 1.6 mmol Cl g^{-1}) was added. The resulting suspension was mechanically stirred for 18 h at 50 °C. Resin **13** was filtered off, washed successively with DMF, CH_2Cl_2, and MeOH, and dried *in vacuo*. *Volhard* titration confirmed the completion of the reaction. IR ν = 3350 (*br.*, OH) cm^{-1}.



Bromination of the Linker (14). Resin **13** (3.63 g, 5.0 mmol) was swelled in dry CH_2Cl_2 (60 mL) for 15 min. PPh_3 (6.56 g, 25.0 mmol) was added. The mixture was cooled down to 0 °C. Slow addition of CBr_4 (8.29 g, 25.0 mmol). The suspension was warmed up to 23 °C and mechanically stirred for 12 h under Ar atmosphere. Resin **14** was filtered off, washed successively with DMF, CH_2Cl_2, and MeOH and dried *in vacuo*. Loading: 1.6 mmol g^{-1} (100%).[7]



Determination of the Loading (*Volhard* Titrations).[7] Dry resin samples (approx. 100 mg, prior and after loading) were heated in pyridine (2 mL) for 2 h at 98 °C. The solutions with the resins were transferred to an *Erlenmeyer* flask with 50 mL of AcOH. A titration according to *Volhard* for bromide was carried out by the addition of saturated ferric ammonium sulfate indicator (5 drops), concentrated nitric acid (5 mL), 0.1 M $AgNO_3$ (5 mL), and toluene (3 mL), followed by back-titration of the excess of Ag^+ with 0.1 M KSCN solution.

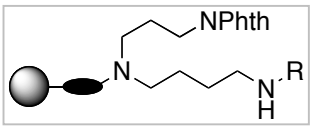
6.3. Synthesis of Solid-Supported Tri- and Tetraamine Derivatives

Loading of Resin 14 with *N*-(2-Nitrophenylsulfonyl)-1,4-diaminobutane 17 to Form Resin 23 (General Procedure). Resin **14** (3.2 mmol, 1.6 g mol^{-1} as determined by *Volhardt* titration[7]) was swelled in NMP (20 mL) for 15 min. DIEA (2.74 mL, 16.0 mmol), *N*-(2-nitrophenylsulfonyl)-1,4-diaminobutane (**17**, 4.37 g, 16.0 mmol) were added, and the suspension was agitated for 24 h at 50 °C. Resin **23** was filtered off, washed successively with NMP, CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 1509 (NO_2), 1362 (SO_2), 1339 (NO_2), 1163 (SO_2) cm^{-1}.

Loading of Resin 14 with *tert*-Butyl *N*-(4-Aminobutyl)carbamate (18) to Form Resin 24. According to the general procedure, resin **14** (0.2 mmol) was loaded with *tert*-butyl *N*-(4-aminobutyl)carbamate (**18**) to give resin **21**. IR ν = 1707 (CO) cm^{-1}.

Loading of Resin 14 with Allyl *N*-(4-Aminobutyl)carbamate (19) to Form Resin 25. According to the general procedure, resin **14** (0.2 mmol) was loaded with allyl *N*-(4-aminobutyl)carbamate (**19**) to give resin **25**. IR ν = 1708 (CO) cm^{-1}.

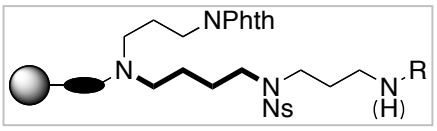
Loading of Resin 14 with 3-Amino-1-propanol (20) to Form Resin 44. According to the general procedure, resin **14** (1.6 mmol) was loaded with 3-amino-1-propanol (**20**) to give resin **44**. IR ν = 3260 (*br.*, OH) cm^{-1}.

Reductive Amination of Resin 23 with 3-Phthalimidopropanal (22) to Form Resin 26 (General Procedure). 

Resin **23** (3.2 mmol) was swelled in DMF (20 mL) for 15 min. 3-Phthalimidopropanal (**22**, 3.26 g, 16.0 mmol) was added, and the suspension was agitated at 23 °C for 2 h. $NaBH(OAc)_3$ (3.38 g, 16.0 mmol) was added, and the suspension was agitated at 23 °C for additional 2 h. Resin **26** was filtered off, washed successively with MeOH, DMF/AcOH (5%), CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 1770 (CO), 1707 (CO), 1509 (NO_2), 1362 (SO_2), 1339 (NO_2), 1163 (SO_2) cm^{-1}.

Reductive Amination of Resin 24 with 3-Phthalimidopropanal (22) to Form Resin 27. According to the general procedure, resin **24** (0.2 mmol) was elongated with 3-phthalimidopropanal (**22**) to give resin **27**. IR ν = 1770 (CO of Phth), 1708 (CO of Boc/Phth) cm^{-1}.

Reductive Amination of Resin 25 with 3-Phthalimidopropanal (22) to Form Resin 28. According to the general procedure, resin **25** (0.2 mmol) was elongated with 3-phthalimidopropanal (**22**) to give resin **28**. IR ν = 1770 (CO of Phth), 1708 (CO of Alloc/Phth) cm^{-1}.

Alkylation of Resin 26 with *N*-(3-Bromopropyl)phthalimide (35) to Form Resin 38 (General Procedure (1)). 

Resin **26** (0.3 mmol) was swelled in DMF (10 mL) at 50 °C for 15 min. Cs_2CO_3 (1.30 g, 4.0 mmol) and *N*-(3-bromopropyl)phthalimide (**35**, 0.72 g, 2.7 mmol) were added, and the suspension was agitated at 50 °C for 24 h. Resin **38** was filtered off, washed successively with DMF, NMP/H_2O (1:1), NMP, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1770 (CO), 1708 (CO), 1509 (NO_2), 1362 (SO_2), 1339 (NO_2), 1163 (SO_2) cm^{-1}.

Alkylation of Resin 26 with *tert*-Butyl *N*-(3-Bromopropyl)carbamate (36) to Form Resin 39. According to the general procedure (1), resin **26** (0.3 mmol) was elongated with *tert*-butyl *N*-(3-bromopropyl)carbamate (**36**) to give resin

39. IR ν = 1770 (CO of Phth), 1707 (CO of Phth/Boc), 1509 (NO_2), 1364 (SO_2), 1339 (NO_2), 1162 (SO_2) cm^{-1}.

Alkylation of Resin 26 with Allyl *N*-(3-Bromopropyl)carbamate (37) to Form Resin 38. According to the general procedure (1), resin **26** (0.6 mmol) was elongated with allyl *N*-(3-bromopropyl)carbamate (**37**) to give resin **40**. IR ν = 1770 (CO of Phth), 1708 (CO of Phth/Alloc), 1509 (NO_2), 1363 (SO_2), 1340 (NO_2), 1163 (SO_2) cm^{-1}.

Alkylation of Resin 44 with *N*-(3-Bromopropyl)phthalimide (35) to Form Resin 45. Resin **44** (1.6 mmol) was swelled in NMP (20 mL) for 15 min. DIEA (1.37 mL, 8.0 mmol), *N*-(3-bromopropyl)phthalimide (**35**, 2.14 g, 8.0 mmol) were added, and the suspension was agitated for 24 h at 50 °C. Resin **45** was filtered off, washed successively with NMP, CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 3350 (*br.*, OH), 1770 (CO), 1708 (CO) cm^{-1}.

Bromination of Resin 45 to Form Resin 46. Resin **45** (1.6 mmol) was swelled in dry CH_2Cl_2 (15 mL). PPh_3 (2.10 g, 8.0 mmol) was added, followed by CBr_4 (2.65 g, 8.0 mmol) dissolved in dry CH_2Cl_2 at 0 °C. The suspension was agitated for 12 h under Ar atmosphere. Resin **46** was filtered off, washed successively with DMF, CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 1770 (CO), 1708 (CO) cm^{-1}.

Alkylation of Resin 46 with *N*-[4-(2-Nitrophenylsulfonamido)butyl]phthalimide (47) to Form Resin 50 (General Procedure (2)). Resin **46** (0.3 mmol) was swelled in DMF (10 mL) at 50 °C for 15 min. Cs_2CO_3 (0.49 g, 1.5 mmol) and *N*-[4-(2-nitrophenylsulfonamido)butyl]phthalimide (**47**, 0.62 g, 1.5 mmol) were added, and the suspension was agitated at 50 °C for 24 h. Resin **50** was filtered off, washed successively with DMF, NMP/H_2O (1:1), NMP, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1770 (CO), 1709 (CO), 1509 (NO_2), 1363 (SO_2), 1339 (NO_2), 1163 (SO_2) cm^{-1}.

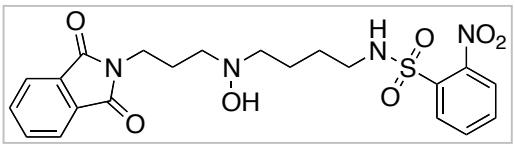
Alkylation of Resin 46 with *tert*-Butyl *N*-[4-(2-Nitrophenylsulfonamido)-butyl]carbamate (48) to Form Resin 51. According to the general procedure (2), resin **46** (1.3 mmol) was elongated with *tert*-butyl *N*-[4-(2-nitrophenylsulfonamido)butyl]carbamate (**48**) to give resin **51**. IR ν = 1770 (CO of Phth), 1707 (CO of Phth/Boc), 1509 (NO_2), 1364 (SO_2), 1162 (SO_2) cm^{-1}.

Alkylation of Resin 46 with Allyl *N*-[4-(2-Nitrophenylsulfonamido)butyl]-carbamate (49) to Form Resin 52. According to the general procedure (2), resin **46** (0.7 mmol) was elongated with allyl *N*-[4-(2-nitrophenylsulfonamido)butyl]carbamate (**49**) to give resin **52**. IR ν = 1770 (CO of Phth), 1707 (CO of Phth/Alloc), 1509 (NO_2), 1363 (SO_2), 1340 (NO_2), 1162 (SO_2) cm^{-1}.

6.4. Liberation of the Tri- and Tetraamine Derivatives from the Resins

Oxidation/*Cope* Elimination (General Procedure): The resin (obtained from 0.3 mmol of resin **14**) was swelled in CH_2Cl_2 (5 mL) for 15 min. *m*-CPBA (0.26 g, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added, and the suspension was agitated for 3 h at 23 °C. The resulting resin was filtered off, washed successively with DMF, MeOH and CH_2Cl_2 and dried *in vacuo*. Toluene (10 mL) was added, and the suspension was heated to 90 °C for 2 h. The resin was filtered off and washed with toluene and CH_2Cl_2. The combined filtrates were evaporated to give a yellow oil, which was purified by HPLC as described below.

***N*-[4-Hydroxy-8-(2-nitrophenylsulfonamido)-4-azaoctyl]phthalimide (32)**



from Resin 29. HPLC (H_2O/ MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **32** as a colourless oil (0.043 g, 0.09 mmol, 30% from resin **14**).

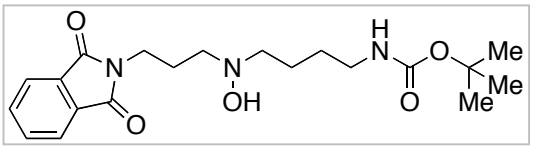
IR ν = 1771 (CO), 1708 (CO), 1364 (SO_2), 1340 (NO_2), 1163 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 8.10–8.09 (m, 1 H, arom. H *o* to NO_2), 7.85–7.83 (m, 3 H, arom. H *o* to SO_2 and 2 arom. CCH of Phth), 7.77–7.76 (m, 2 H, arom. H *p* to NO_2 and arom. H *p* to SO_2), 7.75–7.73 (m, 2 H, 2 arom. CCHC*H* of Phth), 3.81 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, PhthNCH_2), 3.39–3.20 (m, 4 H, CH_2NOH-CH_2), 3.13–3.09 (m, 2 H, NsNHCH_2), 2.24–2.11 (m, 2 H, PhthNCH_2CH_2), 1.93–1.78 (m, 2 H, NsNHCH_2CH_2CH_2), 1.65 (quint, $^3J_{H,H}$ = 7.2 Hz, 2 H, NsNHCH_2CH_2) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.5 (s, 2 CO), 147.9 (s, arom. CNO_2), 134.4 (d, 2 arom. CCHCH of Phth), 133.8 (d, arom. CH *p* to NO_2), 133.1 (s, arom. CSO_2), 132.9 (d, arom. CH *p* to SO_2), 131.7 (s, 2 arom. C of Phth), 131.1 (d, arom. CH *o* to NO_2), 125.5 (d, arom. CH *o* to SO_2), 123.6 (d, 2 arom. CCH of Phth), 59.2, 57.1 (2 t, CH_2NOHCH_2), 42.9 (t, NsNHCH_2), 34.6 (t, PhthNCH_2), 26.4 (t, NsNHCH_2CH_2), 23.6 (t, PhthCH_2CH_2), 20.6 (t, NsNHCH_2CH_2CH_2) ppm.

ESI-MS *m*/*z* = 477.2 (100, $[M+H]^+$).

HR-MS: calcd. for $C_{21}H_{25}N_4O_7S_1$ 477.1444; found 477.1449.

***tert*-Butyl *N*-(5-Hydroxy-8-phthalimido-5-azaoctyl)carbamate (33) from Resin 30.**  HPLC (H_2O/MeCN/ TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **33** as a colourless oil (0.025 g, 0.06 mmol, 32% from resin **14**).

IR ν = 1773 (CO of Phth), 1710 (CO of Boc/Phth) cm^{-1}.

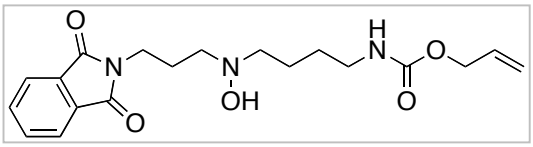
^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 7.90–7.88 (m, 2 H, 2 arom. CCH of Phth), 7.79–7.77 (m, 2 H, 2 arom. CCHC*H* of Phth), 4.67 (br. s, 1 H, NH), 3.85 (t, $^3J_{H,H}$ = 6.2 Hz, 2 H, PhthNCH_2), 3.35–3.26 (m, 4 H, CH_2NOHCH_2), 3.19–3.14 (m, 2 H, BocNHCH_2), 2.28–2.15 (m, 2 H, PhthNCH_2CH_2), 1.95–1.75 (m, 2 H, BocNHCH_2CH_2CH_2), 1.59 (quint, $^3J_{H,H}$ = 7.1 Hz, 2 H, BocNHCH_2CH_2), 1.45 (s, 9 H, Me_3C) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.3 (s, 2 CO of Phth), 156.1 (s, CO of Boc), 134.4 (d, 2 arom. CCHCH of Phth), 131.8 (s, 2 arom. C of Phth), 123.6 (d, 2 arom. CCH of Phth), 79.6 (s, Me_3C), 59.2, 57.0 (2 t, 2 CH_2), 39.4 (t, BocNHCH_2), 34.8 (t, PhthNCH_2), 28.3 (*q*, *Me*$_3$C), 27.0 (t, BocNHCH_2CH_2), 26.6 (t, PhthNCH_2CH_2), 20.6 (t, BocNHCH_2CH_2CH_2) ppm.

ESI-MS *m*/*z* = 392.3 (100, [*M*+H]$^+$), 336.3 (13).

HR-MS: calcd. for $C_{20}H_{30}N_3O_5$ 392.2185; found 392.2186.

Allyl *N*-(5-Hydroxy-8-phthalimido-5-azaoctyl)carbamate (34) from Resin 31.



HPLC (H_2O/MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **34** as a colourless oil (0.022 g, 0.06 mmol, 30% from resin **14**).

IR ν = 1771 (CO of Phth), 1705 (CO of Alloc/Phth) cm^{-1}.

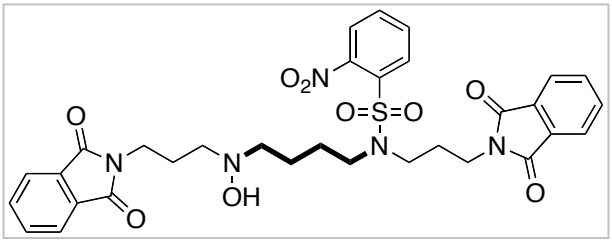
^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 7.88–7.84 (m, 2 H, 2 arom. CCH of Phth), 7.77–7.72 (m, 2 H, 2 arom. CCHC*H* of Phth), 5.90 (ddt, $^3J_{H,H\text{-}trans}$ = 17.1, $^3J_{H,H\text{-}cis}$ = 10.6 Hz, $^3J_{H,H\text{-}vic}$ = 5.3 Hz, 1 H, CH_2=C*H*), 5.30 (dd, $^3J_{H,H\text{-}trans}$ = 17.1, $^2J_{H,H\text{-}gem}$ = 1.5 Hz, 1 H, CH_2=CH), 5.21 (d, $^3J_{H,H\text{-}cis}$ = 10.6 Hz, 1 H, CH_2=CH), 4.87 (br. s, 1 H, NH), 4.54 (d, $^3J_{H,H}$ = 5.3 Hz, 2 H, OCH_2), 3.83 (t, $^3J_{H,H}$ = 6.2 Hz, 2 H, PhthNCH_2), 3.32–3.22 (m, 4 H, CH_2NOHCH_2), 3.19–3.22 (m, 2 H, AllocNH-CH_2), 2.22–2.10 (m, 2 H, PhthNCH$_2$CH_2), 1.94–1.74 (m, 2 H, AllocNHCH$_2$-CH$_2$CH_2), 1.60 (quint, $^3J_{H,H}$ = 7.0 Hz, 2 H, AllocNHCH$_2$CH_2) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.4 (s, 2 CO of Phth), 156.4 (s, CO of Alloc), 134.3 (d, 2 arom. CCHCH of Phth), 132.8 (d, CH_2=*C*H), 131.8 (s, 2 arom. C of Phth), 123.6 (d, 2 arom. *C*CH of Phth), 117.8 (t, *C*H_2=CH), 65.7 (t, CH_2=CH*C*H_2), 59.2, 57.1 (2 t, 2 CH_2), 39.9 (t, AllocNH*C*H_2), 34.8 (t, PhthN*C*H_2), 26.9 (t, NHCH$_2$*C*H$_2$), 23.6 (t, PhthNCH$_2$*C*H$_2$), 20.6 (t, NHCH$_2$CH$_2$*C*H$_2$) ppm.

ESI-MS *m*/*z* = 376.3 (100, [*M*+H]$^+$), 360.3 (10).

HR-MS: calcd. for $C_{19}H_{26}N_3O_5$ 376.1872; found 376.1871.

[4-Hydroxy-9-(2-nitrophenylsulfonyl)-4,9-diazadodecane]-1,12-diphthalimide (41) from Resin 38. HPLC (H_2O/MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **41** as a colourless solid (0.059 g, 0.09 mmol, 30% from resin **14**).



Mp 67–70 °C.

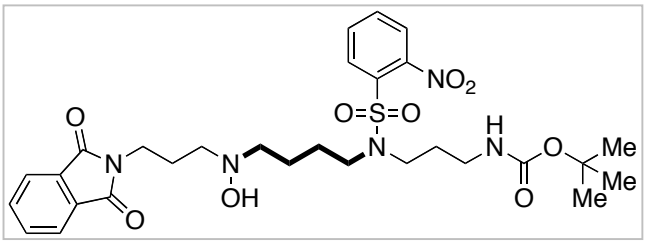
IR ν = 1769 (CO), 1708 (CO), 1370 (SO_2), 1341 (NO_2), 1161 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 7.96 (dd, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 1.3 Hz, 1 H, arom. H *o* to NO_2), 7.86–7.81 (m, 4 H, 4 arom. CCH of Phth), 7.75–7.72 (m, 4 H, 4 arom. CCHC*H* of Phth), 7.71 (app. td, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 1.3 Hz, 1 H, arom. H *p* to NO_2), 7.66 (app. td, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 1.3 Hz, 1 H, arom. H *p* to SO_2), 7.61 (dd, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 1.3 Hz, 1 H, arom. H *o* to SO_2), 3.83 (t, $^3J_{H,H}$ = 6.2 Hz, 2 H, PhthNCH_2(CH_2)$_2$NOH), 3.67 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, PhthNCH_2(CH_2)$_2$NNs), 3.38–3.34 (m, 4 H, CH_2NNsCH_2), 3.33–3.28 (m, 4 H, CH_2NOHCH_2), 2.23–2.16 (m, 2 H, PhthNCH$_2$CH_2CH$_2$OH), 1.92 (quint, $^3J_{H,H}$ = 7.5 Hz, 2 H, NsNCH$_2$-CH_2CH$_2$NPhth), 1.86–1.78 (m, 2 H, HONCH$_2$CH_2(CH_2)$_2$NNs), 1.77–1.71 (m, 2 H, HON(CH_2)$_2$CH_2CH$_2$NNs) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.3, 168.2 (2 s, 2×2 CO of Phth), 148.0 (s, arom. CNO_2), 134.3, 134.1 (2 d, 2×2 arom. CCHCH of Phth), 133.8 (d, arom. CH *p* to NO_2), 132.8 (s, arom. CSO_2), 131.9 (d, arom. CH *p* to SO_2), 131.8 (s, 4 arom. C of Phth), 130.7 (d, arom. CH *o* to NO_2), 124.3 (d, arom. CH *o* to CSO_2), 123.5, 123.3 (2 d, 2×2 arom. CCH of Phth), 58.9, 56.9 (2 t, CH_2NNsCH_2), 47.1, 45.8 (2 t, CH_2NOHCH_2), 35.6 (t, PhthNCH_2(CH_2)$_3$NNs), 34.7 (t, PhthN-CH_2(CH_2)$_2$NOH), 27.4 (t, HONCH$_2$CH$_2$CH$_2$CH$_2$NNs), 25.6 (t, PhthNCH$_2$CH$_2$-CH$_2$NNs), 23.6 (t, PhthNCH$_2$CH$_2$CH$_2$NOH), 20.4 (t, HONCH$_2$CH$_2$(CH_2)$_2$NNs) ppm.

ESI-MS *m*/*z* = 702.3 (12, [*M*+K]$^+$), 686.3 (28, [*M*+Na]$^+$), *m*/*z* = 664.4 (100, [*M*+H]$^+$), 646.3 (9).

HR-MS: calcd. for $C_{32}H_{34}N_5O_9S_1$ 664.2077; found 664.2086.

***tert*-Butyl *N*-[9-Hydroxy-4-(2-nitrophenylsulfonyl)-12-phthalimido-4,9-diazadodecyl]carbamate (42) from Resin 39.**  HPLC (H_2O/MeCN/TFA 55:45:0.1, 25 mL min^{-1}, λ = 280 nm) gave **42** as a colourless oil (0.053 g, 0.08 mmol, 28% from resin **14**).

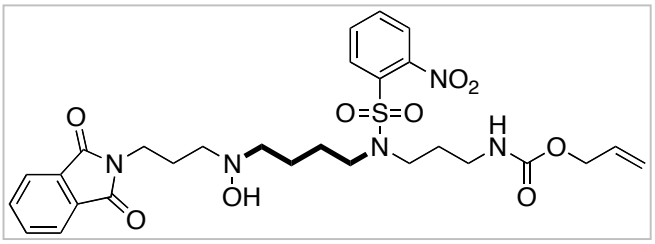
IR ν = 1773 (CO of Phth), 1712 (CO of Phth/Boc), 1367 (SO_2), 1345 (NO_2), 1163 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 7.96 (d, $^3J_{H,H}$ = 7.2 Hz, 1 H, arom. H *o* to NO_2), 7.87–7.86 (m, 2 H, 2 arom. CC of Phth), 7.77–7.75 (m, 2 H, 2 arom. CCHC*H* of Phth), 7.74 (ap. td, $^3J_{H,H}$ = 7.7, $^4J_{H,H}$ = 1.6 Hz, 1 H, arom. H *p* to NO_2), 7.71 (ap. td, $^3J_{H,H}$ = 7.6, $^4J_{H,H}$ = 1.6 Hz, 1 H, arom. H *p* to SO_2), 7.65 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, arom. H *o* to SO_2), 3.82 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, PhthNCH_2), 3.35–3.23 (m, 10 H, CH_2NOHCH_2, CH_2NNsCH_2, BocNHCH_2), 2.18–2.11 (m, 2 H, PhthNCH_2CH_2), 1.82–1.63 (m, 6 H, HONCH_2(CH_2)$_2$CH_2NNs, BocNHCH_2CH_2), 1.42 (s, 9 H, Me_3C) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.5 (s, 2 CO of Phth), 156.2 (s, CO of Boc), 147.9 (s, arom. CNO_2), 134.4 (d, 2 arom. CCHCH of Phth), 133.8 (d, arom. CH *p* to NO_2), 132.6 (s, arom. CSO_2), 131.9 (d, arom. CH *p* to SO_2), 131.6 (s, 2 arom. C of Phth), 130.4 (d, arom. CH *o* to NO_2), 124.3 (d, arom. CH *o* to SO_2), 123.6 (d, 2 arom. CCH of Phth), 79.5 (s, Me_3C), 58.9, 57.0 (2 t, CH_2NNsCH_2), 46.8, 45.4 (2 t, CH_2NOHCH_2), 37.2 (t, BocNHCH_2), 34.6 (t, PhthNCH_2), 28.5 (t, CH_2), 28.3 (q, *Me*$_3$C), 25.2 (t, BocNHCH$_2$CH_2), 23.5 (t, PhthNCH$_2$CH_2), 20.4 (t, CH_2) ppm.

ESI-MS *m*/*z* = 672.3 (10, [*M*+K]$^+$), 656.3 (10, [*M*+Na]$^+$), 634.3 (100, [*M*+H]$^+$).

HR-MS: calcd. for $C_{29}H_{40}N_5O_9S_1$ 634.2547; found 634.2543.

Allyl *N*-[9-Hydroxy-4-(2-nitrophenylsulfonyl)-12-phthalimido-4,9-diazadodecyl]carbamate (43) from Resin 40. 

HPLC (H_2O/MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **43** as a colouless oil (0.022 g, 0.06 mmol, 30% from resin **14**).

IR ν = 1772 (CO of Phth), 1711 (CO of Phth/Alloc), 1371 (SO_2), 1142 (SO_2) cm^{-1}.

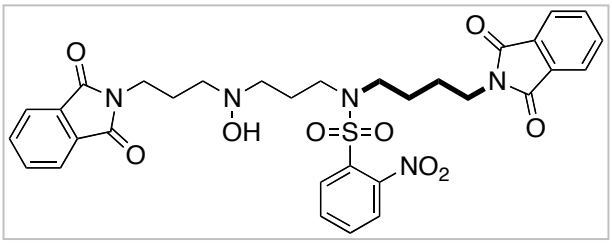
^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 7.96 (dd, $^3J_{H,H}$ = 7.4, $^4J_{H,H}$ = 1.7 Hz, 1 H, arom. H *o* to NO_2), 7.88–7.86 (m, 2 H, 2 arom. CCH of Phth), 7.77–7.76 (m, 2 H, 2 arom. CCHC*H* of Phth), 7.75 (ap. td, $^3J_{H,H}$ = 7.4, $^4J_{H,H}$ = 1.5 Hz, 1 H, arom. H *p* to NO_2), 7.72 (ap. td, $^3J_{H,H}$ = 7.5, $^4J_{H,H}$ = 1.5 Hz, 1 H, arom. H *p* to SO_2), 7.66 (dd, $^3J_{H,H}$ = 7.5, $^4J_{H,H}$ = 1.5 Hz, 1 H, arom. H *o* to SO_2), 5.90 (ddt, $^3J_{H,H\text{-}trans}$ = 17.3, $^3J_{H,H\text{-}cis}$ = 10.7, $^3J_{H,H\text{-}vic}$ = 5.5 Hz, 1 H, CH_2=C*H*), 5.30 (dd, $^3J_{H,H\text{-}trans}$ = 17.1, $^2J_{H,H\text{-}gem}$ = 1.3 Hz, 1 H, CH_2=CH), 5.21 (dd, $^3J_{H,H\text{-}cis}$ = 10.7, $^2J_{H,H\text{-}gem}$ = 1.3 Hz, 1 H, CH_2=CH), 4.53 (d, $^3J_{H,H\text{-}vic}$ = 5.5 Hz, 2 H, OCH_2), 3.83 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, PhthNCH_2), 3.36–3.21 (m, 10 H, CH_2NOHCH_2, CH_2NNsCH_2, AllocNHCH_2), 2.19–2.14 (m, 2 H, PhthNCH_2CH_2), 1.82–1.73 (m, 4 H, HONCH_2CH_2CH_2CH_2NNs), 1.72–1.65 (m, 2 H, AllocNHCH_2CH_2) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.4 (s, 2 CO of Phth), 156.4 (s, CO of Alloc), 147.8 (s, arom. CNO_2), 134.4 (d, 2 arom. CCHCH of Phth), 133.9 (d, arom. CH *p* to NO_2), 132.8 (d, CH_2=*C*H), 132.5 (s, arom. CSO_2), 131.9 (d, arom. CH *p* to SO_2) 131.6 (s, 2 arom. C of Phth), 130.5 (d, arom. CH *o* to NO_2), 124.4 (d, arom. CH *o* to SO_2), 123.6 (d, 2 arom. C*C*H of Phth), 117.7 (t, *C*H_2=CH), 65.6 (t, CH_2=CH*C*H_2), 58.9, 57.1 (2 t, CH_2NNsCH_2), 46.9, 45.3 (2 t, CH_2NOHCH_2), 37.5 (t, AllocNH*C*H_2), 34.5 (t, PhthN*C*H_2), 28.3 (t, *C*H_2), 25.3 (t, AllocNHCH_2-*C*H_2), 23.5 (t, PhthNCH_2*C*H_2), 20.4 (t, *C*H_2) ppm.

ESI-MS *m*/*z* = 618.3 (100, $[M+H]^+$).

HR-MS: calcd. for $C_{28}H_{36}N_5O_9S_1$ 618.2234; found 618.2228.

[4-Hydroxy-8-(2-nitrophenylsulfonyl)-4,8-diazadodecane]-1,12-diphthal-



imide (53) from Resin 50. HPLC (H_2O/MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **53** as a colourless solid (0.040 g, 0.06 mmol, 21% from resin **14**).

Mp 66–69 °C.

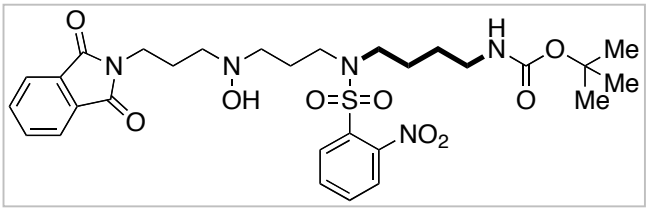
IR ν = 1771 (CO of Phth), 1709 (CO of Phth/Alloc), 1371 (SO_2), 1155 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 8.26–8.25 (m, 1 H, arom. H *o* to NO_2), 7.85–7.83 (m, 4 H, 4 arom. CCH of Phth), 7.75–7.68 (m, 6H, 4 arom. CCHC*H* of Phth, 1 arom. H *p* to NO_2, 1 H, arom. H *p* to SO_2), 7.56–7.55 (m, 1 H, arom. H *o* to SO_2), 3.85–3.82 (m, 2 H, PhthNCH_2(CH_2)$_2$NOH), 3.68–3.64 (m, 2 H, PhthNCH_2(CH_2)$_3$NNs), 3.53–3.22 (m, 8 H, CH_2NOHC$H_2$$CH_2CH_2$NNsC$H_2$), 2.28–2.03 (m, 2 H, PhthNCH_2C$H_2$$CH_2$NOH), 2.08–2.03 (m, 2 H, HONCH_2-C$H_2$$CH_2$NNs), 1.70–1.65 (m, 2 H, NsNC$H_2$$CH_2CH_2$$CH_2$NPhth), 1.61–1.51 (m, 2 H, NsNCH_2C$H_2$$CH_2$$CH_2$NPhth) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.4, 18.3 (2 s, 2×2 CO of Phth), 147.8 (s, arom. CNO_2), 134.2, 134.1 (2 d, 2×2 arom. CCHCH of Phth), 133.8 (d, arom. CH *p* to NO_2), 132.2 (s, arom. CSO_2), 132.0 (d, arom. CH *p* to SO_2), 131.8, 131,7 (2 s, 2×2 arom. C of Phth), 131.3 (d, arom. CH *o* to NO_2), 123.9 (d, arom. CH *o* to CSO_2), 123.5, 123.3 (2 d, 2×2 arom. C*C*H of Phth), 57.8, 56.9 (2 t, CH_2NNsCH_2), 47.7, 44.3 (2 t, CH_2NOHCH_2), 36.9 (t, PhthNCH_2(CH_2)$_3$NNs), 34.7 (t, PhthNCH_2(CH_2)$_2$NOH), 25.6 (2 t, PhthNC$H_2$$CH_2$$CH_2$$CH_2$NNs), 23.4 (t, PhthNC$H_2$$CH_2$$CH_2$NOH), 22.7 (t, HONC$H_2$$CH_2$$CH_2$NNs) ppm.

ESI-MS *m*/*z* = 702.2 (12, [*M*+K]$^+$), 686.2 18, [*M*+Na]$^+$), 664.3 (100, [*M*+H]$^+$).

HR-MS: calcd. for $C_{32}H_{34}N_5O_9S_1$ 664.2077; found 664.2074.

***tert*-Butyl *N*-[9-Hydroxy-5-(2-nitrophenylsulfonyl)-12-phthalimido-5,9-diazadodecyl]carbamate (54) from Resin 51.**  HPLC (H_2O/MeCN/TFA 55:45:0.1, 25 mL min^{-1}, λ = 280 nm) gave 54 as a colourless oil (0.032 g, 0.05 mmol, 18% from resin 14).

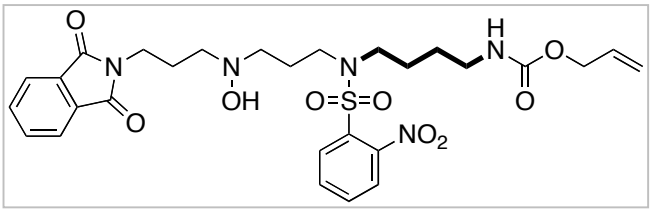
IR ν = 1772 (CO of Phth), 1710 (CO of Phth/Boc), 1398 (SO_2), 1345 (NO_2), 1144 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 8.26 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, arom. H *o* to NO_2), 7.85–7.84 (m, 2 H, 2 arom. CCH of Phth), 7.75–7.70 (m, 4 H, 2 arom. CCHC*H* of Phth, arom. H *p* to NO_2, arom. H *p* to SO_2), 7.62 (dd, $^3J_{H,H}$ = 7.4, $^4J_{H,H}$ = 1.5 Hz, 1 H, arom. H *o* to SO_2), 4.68 (app. t, $^3J_{H,H}$ = 6.0 Hz, 1 H, BocNH), 3.87–3.79 (m, 2 H, PhthNCH_2), 3.67–3.62 (m, 1 H, HONCH_2CH_2CH_2NNs), 3.52–3.47 (m, 1 H, HONCH_2CH_2CH_2NNs), 3.40–3.23 (m, 6 H, CH_2NOHCH_2, NsNCH_2(CH_2)$_3$NHBoc), 3.10 (app. td, $^3J_{H,H}$ = 6.6, $^3J_{H,H}$ = 6.0 Hz, 2 H, BocNHCH_2), 2.27–2.16 (m, 2 H, PhthNCH_2CH_2), 2.07–2.03 (m, 2 H, HONCH_2CH_2CH_2NNs), 1.62–1.51 (m, 2 H, NsNCH_2CH_2CH_2CH_2NHBoc), 1.50–1.43 (m, 2 H, NsNCH_2CH_2CH_2CH_2NHBoc), 1.41 (s, 9 H, Me_3C) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.4 (s, 2 CO of Phth), 156.1 (s, CO of Boc), 147.8 (s, arom. CNO_2), 134.3 (d, 2 arom. CCHCH of Phth), 133.8 (d, arom. CH *p* to NO_2), 132.3 (s, arom. CSO_2), 132.1 (d, arom. CH *p* to SO_2), 131.7 (s, 2 arom. C of Phth), 131.3 (d, arom. CH *o* to NO_2), 124.0 (d, arom. CH *o* to SO_2), 123.5 (d, 2 arom. CCH of Phth), 57.9 (t, NsNCH_2(CH_2)$_3$NPhth), 56.9 (t, HON(CH_2)$_2$CH_2), 47.9 (t, PhthN(CH_2)$_2$CH_2NOH), 44.2 (t, HONCH_2(CH_2)$_2$NNs), 39.6 (t, BocNHCH_2), 34.6 (t, PhthNCH_2), 28.3 (q, *Me*$_3$C), 27.1 (t, BocNHCH_2-CH_2), 25.7 (t, BocNHCH_2CH_2CH_2), 23.5 (t, PhthNCH_2CH_2), 22.6 (t, NOHCH_2-CH_2CH_2NNs) ppm.

ESI-MS *m*/*z* = 634.3 (100, [*M*+H]$^+$).

HR-MS: calcd. for $C_{29}H_{40}N_5O_9S_1$ 634.2547; found 634.2552.

Allyl *N*-[9-Hydroxy-5-(2-nitrophenylsulfonyl)-12-phthalimido-5,9-diazadodecyl] carbamate (55) from Resin 52. 

HPLC (H_2O/MeCN/TFA 60:40:0.1, 25 mL min^{-1}, λ = 280 nm) gave **55** as a colourless oil (0.037 g, 0.06 mmol, 20% from resin **14**). IR ν = 1770 (CO of Phth), 1708 (CO of Phth/Alloc), 1368 (SO_2), 1345 (NO_2), 1142 (SO_2) cm^{-1}.

^{1}H-NMR (600 MHz, $CDCl_3$, 278 K) δ = 8.22 (dd, $^3J_{H,H}$ = 7.2, $^4J_{H,H}$ = 1.5 Hz, 1 H, arom. H *o* to NO_2), 7.86–7.84 (m, 2 H, 2 arom. CCH of Phth), 7.75–7.70 (m, 3 H, 2 arom. CCHC*H* of Phth, 1 arom. H *p* to NO_2,), 7.71 (dd, $^3J_{H,H}$ = 7.6, $^4J_{H,H}$ = 1.7 Hz, 1 H, arom. H *p* to SO_2), 7.62 (dd, $^3J_{H,H}$ = 7.0, $^4J_{H,H}$ = 2.0 Hz, 1 H, arom. H *o* to SO_2), 5.89 (ddt, $^3J_{H,H\text{-}trans}$ = 17.2, $^3J_{H,H\text{-}cis}$ = 10.4, $^3J_{H,H\text{-}vic}$ = 5.4 Hz, 1 H, CH_2=C*H*), 5.28 (dd, $^3J_{H,H\text{-}trans}$ = 17.2, $^2J_{H,H\text{-}gem}$ = 1.5 Hz, 1 H, CH_2=CH), 5.20 (dd, $^3J_{H,H\text{-}cis}$ = 10.5, $^2J_{H,H\text{-}gem}$ = 1.4 Hz, 1 H, CH_2=CH), 4.52 (dt, $^3J_{H,H\text{-}vic}$ = 5.4, $^4J_{H,H}$ = 1.3 Hz , 2 H, OCH_2), 3.87–3.79 (m, 2 H, PhthNCH_2), 3.64–3.59 (m, 1 H, HONCH_2-CH_2CH_2NNs), 3.52–3.47 (m, 1 H, HONCH_2CH_2CH_2NNs), 3.41–3.25 (m, 6 H, CH_2NOHCH_2, NsNCH_2(CH_2)$_3$NHAlloc), 3.18 (td, $^3J_{H,H}$ = 6.5, $^3J_{H,H}$ = 6.5 Hz, 2 H, AllocNHCH_2), 2.24–2.19 (m, 2 H, PhthNCH_2CH_2), 2.08–2.04 (m, 2 H, HONCH_2-CH_2CH_2NNs), 1.62–1.56 (m, 2 H, NsNCH_2CH_2CH_2CH_2NHAlloc), 1.52–1.48 (m, 2 H, NsNCH_2CH_2CH_2CH_2NHAlloc) ppm.

^{13}C-NMR (150 MHz, $CDCl_3$, 278 K) δ = 168.4 (s, 2 CO of Phth), 156.6 (s, CO of Alloc), 148.0 (s, arom. CNO_2), 134.5 (d, 2 arom. CCHCH of Phth), 134.1 (d, arom. CH *p* to NO_2), 133.0 (d, CH_2=CH), 132.4 (s, arom. CSO_2), 132.3 (d, arom. CH *p* to SO_2), 131.9 (s, 2 arom. C of Phth), 131.4 (d, arom. CH *o* to NO_2), 124.2 (d, arom. CH *o* to SO_2), 123.7 (d, 2 arom. CCH of Phth), 117.9 (t, CH_2=CH), 65.7 (t, CH_2=CHCH_2), 58.1 (t, NsNCH_2(CH_2)$_3$NPhth), 57.1 (t, HON(CH_2)$_2$$CH_2$), 48.2 (t, PhthN($CH_2$)$_2$$CH_2$NOH), 44.7 (t, HONC$H_2$($CH_2$)$_2$NNs), 40.3 (t, AllocNHC$H_2$), 34.8 (t, PhthNC$H_2$), 27.2 (t, AllocNHC$H_2CH_2$), 26.0 (t, AllocNHC$H_2CH_2CH_2$), 23.7 (t, PhthNC$H_2CH_2$), 22.9 (t, HONC$H_2CH_2CH_2$NNs) ppm.

ESI-MS *m*/*z* = 618.2 (100, [*M*+H]$^+$).

HR-MS: calcd. for $C_{28}H_{36}N_5O_9S_1$ 664.2234; found 618.2232.

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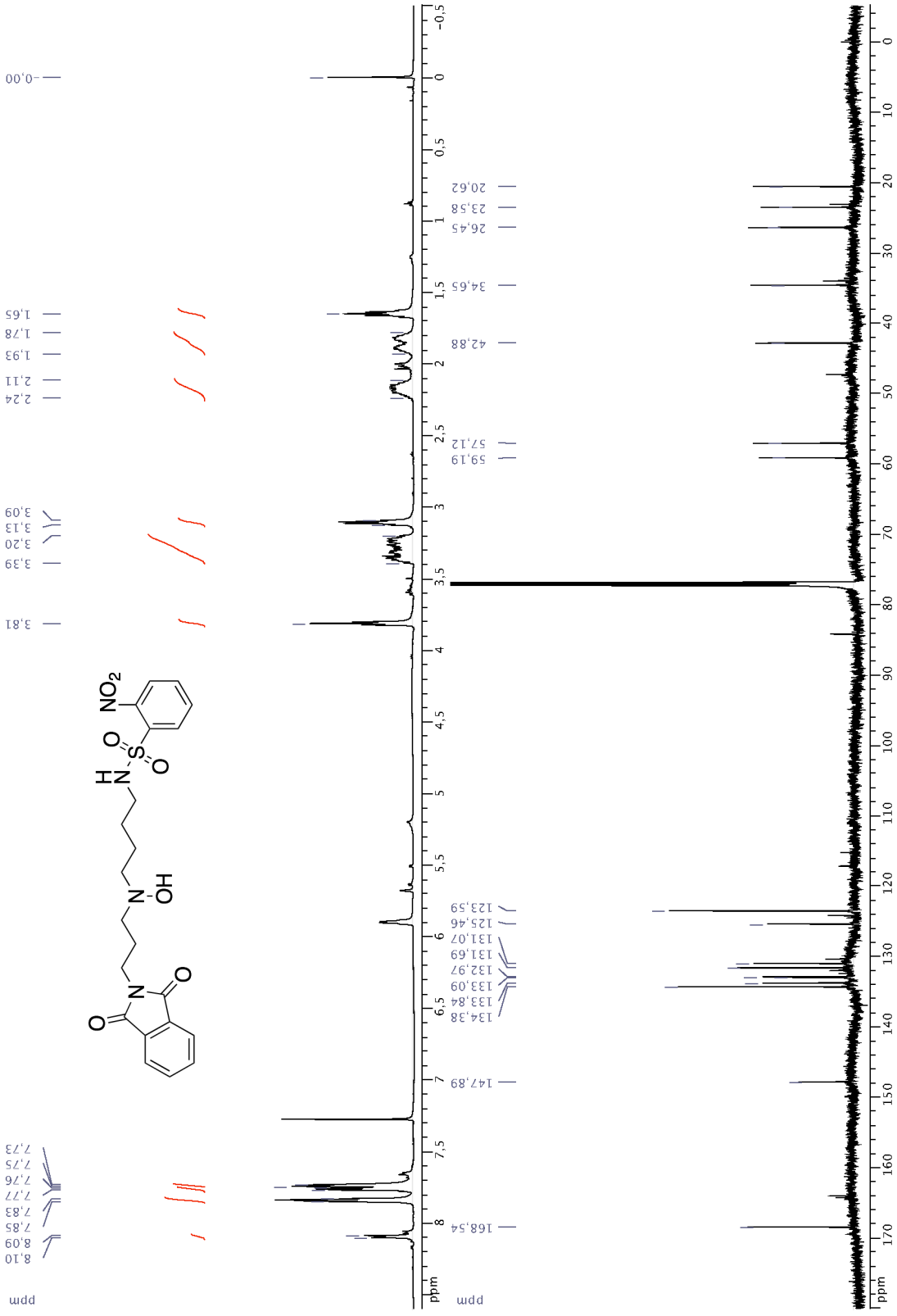
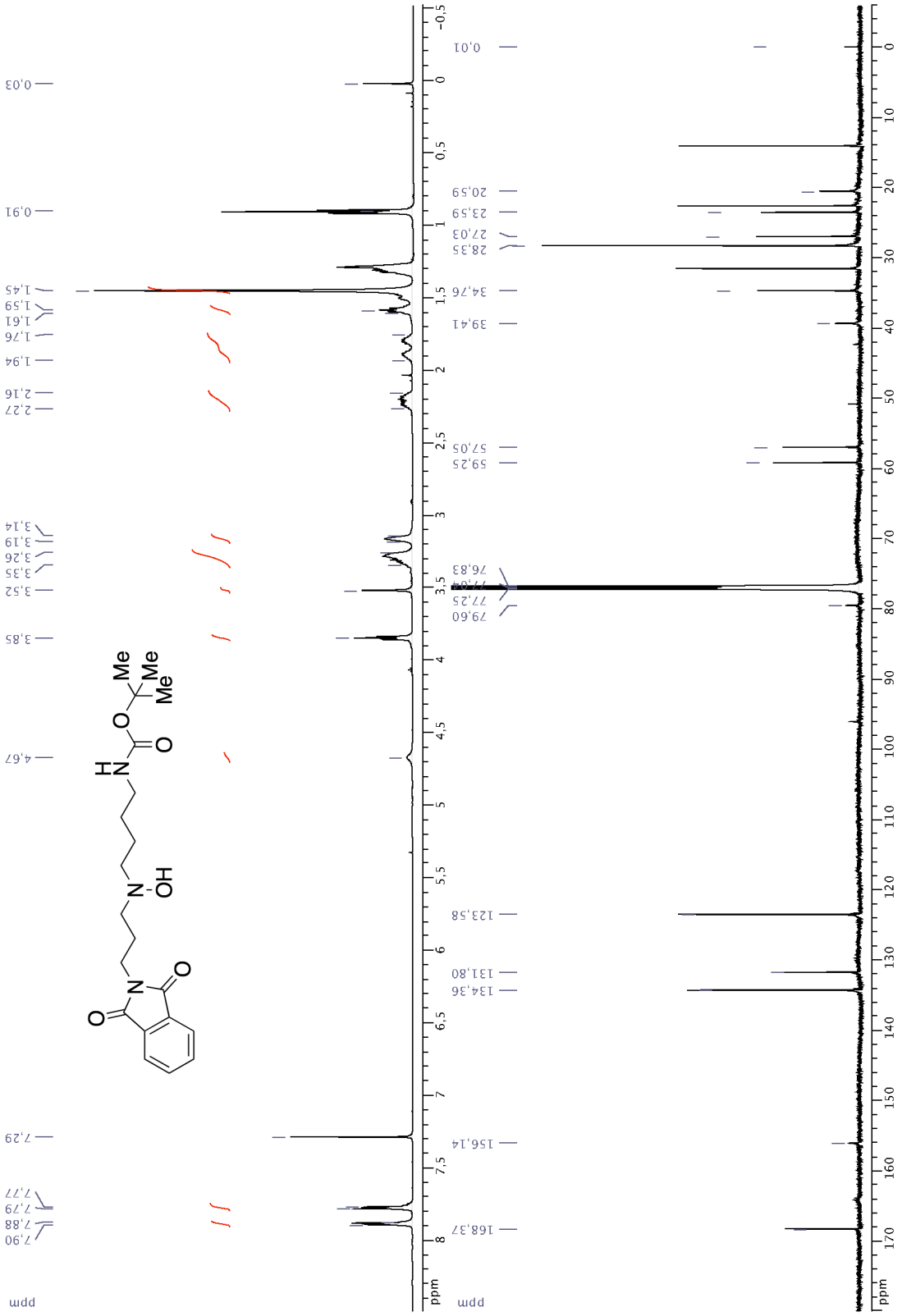
APPENDICES — NMR OF FINAL COMPOUNDS

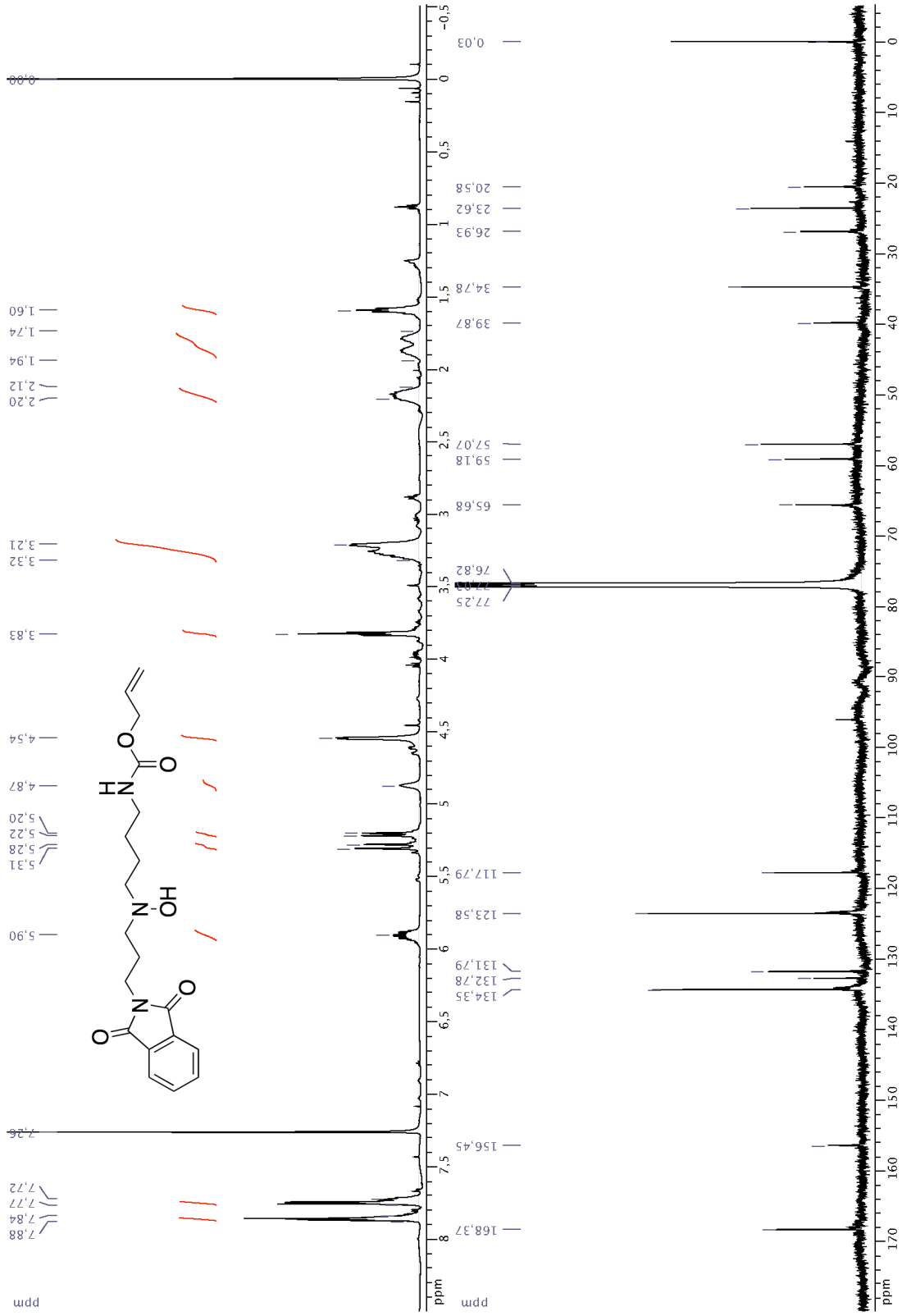
Triamine Derivative **32**



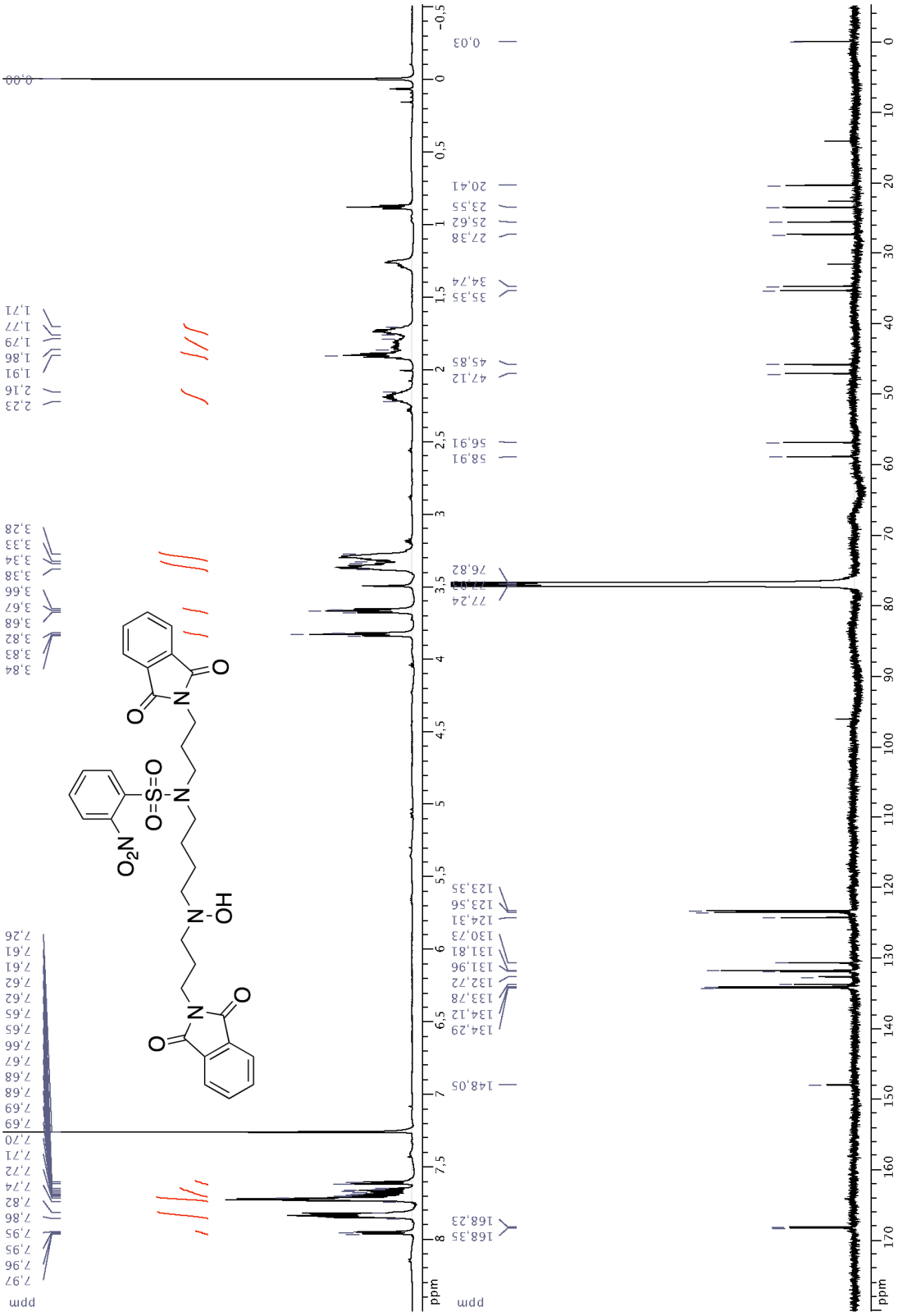
Triamine Derivative **33**



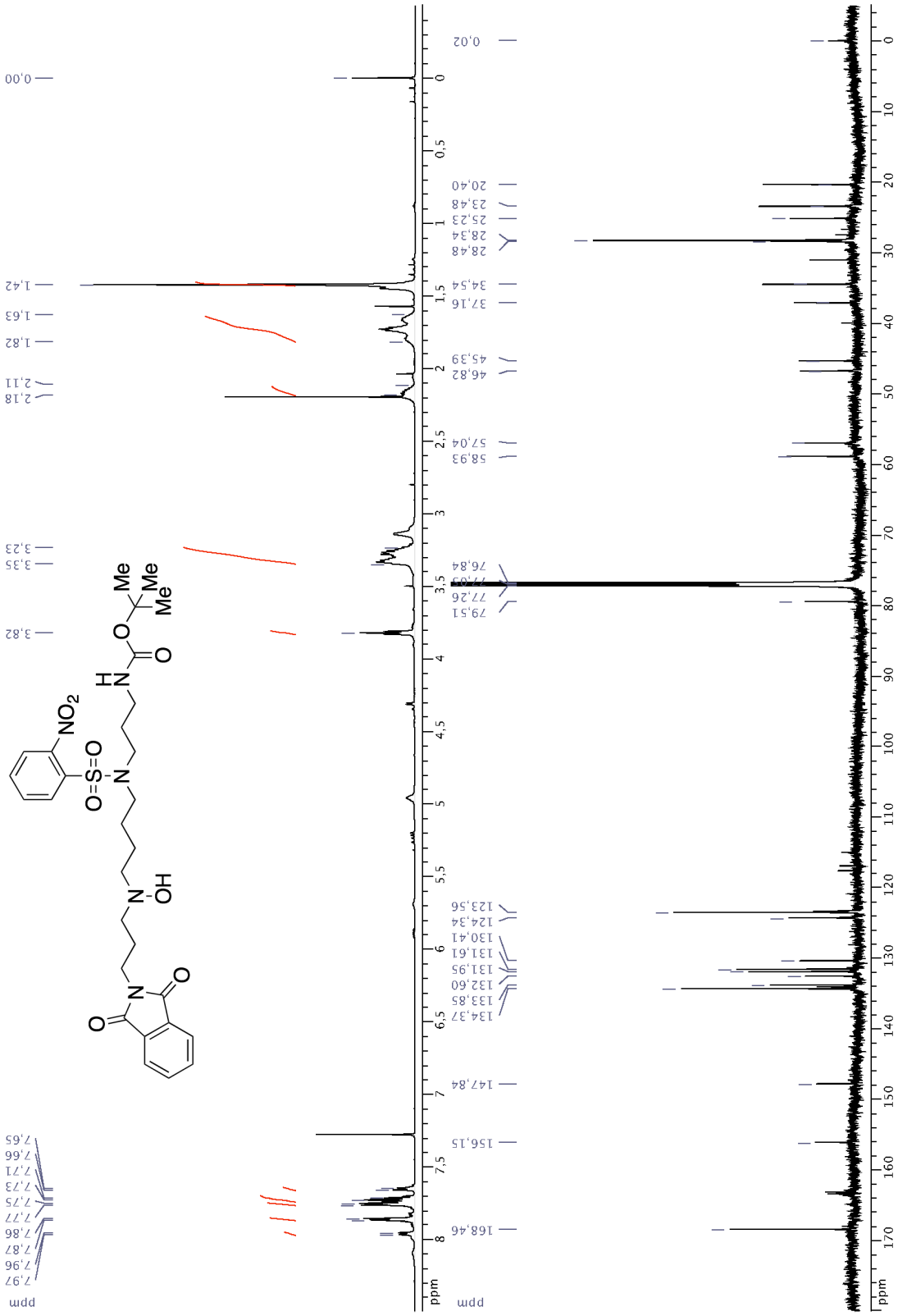
Triamine Derivative **34**



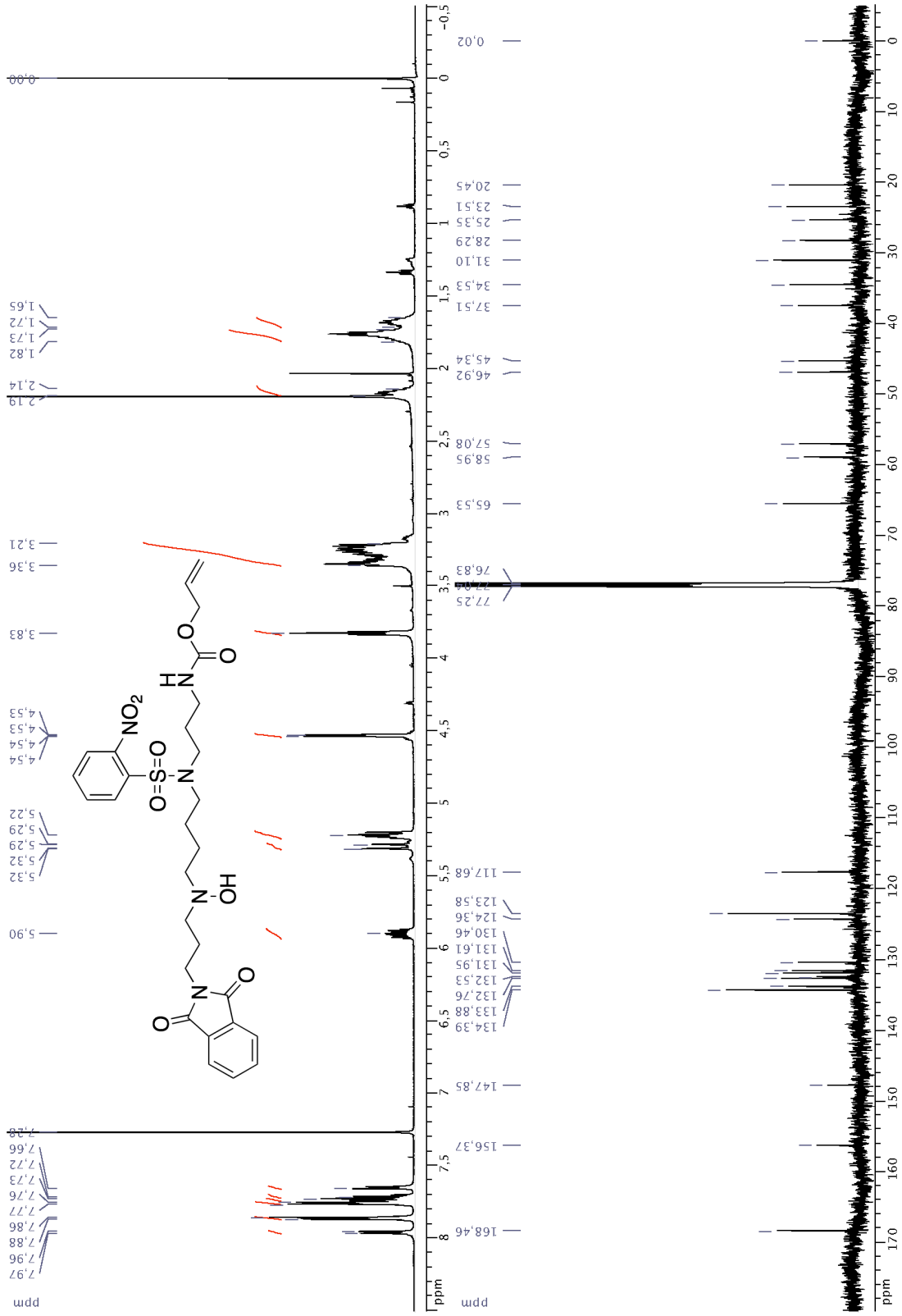
Tetraamine Derivative **41**



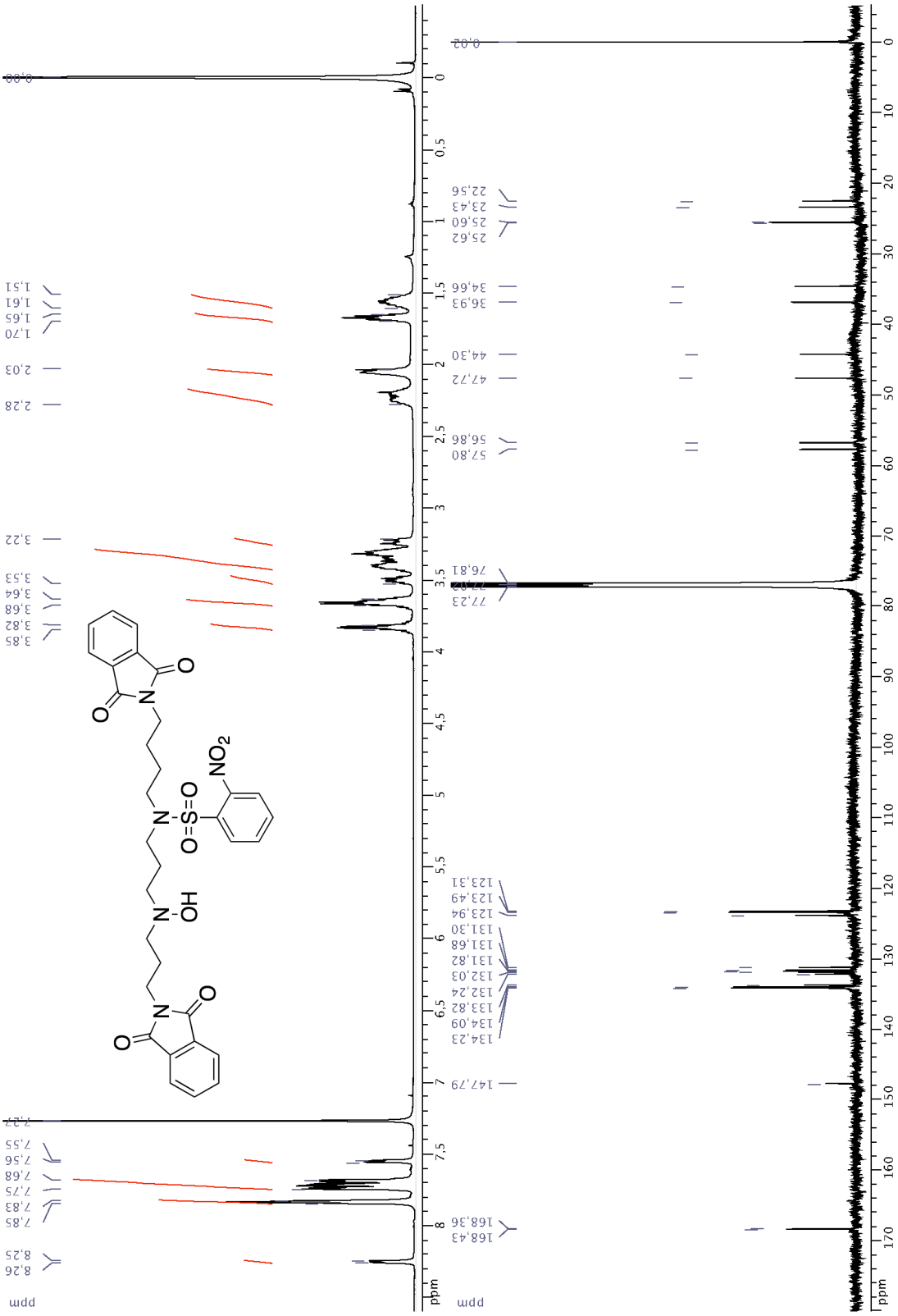
Tetraamine Derivative **42**



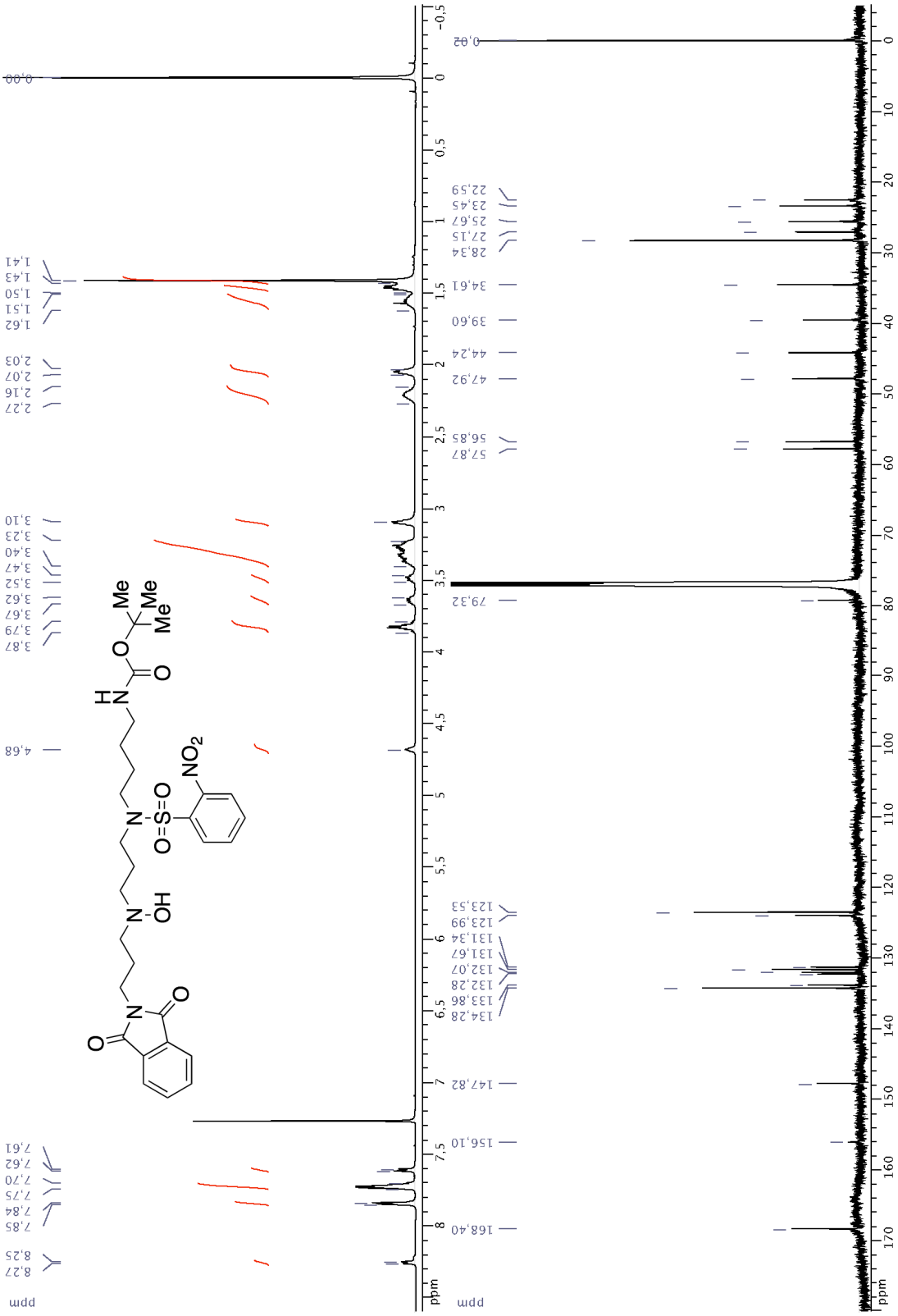
Tetraamine Derivative **43**



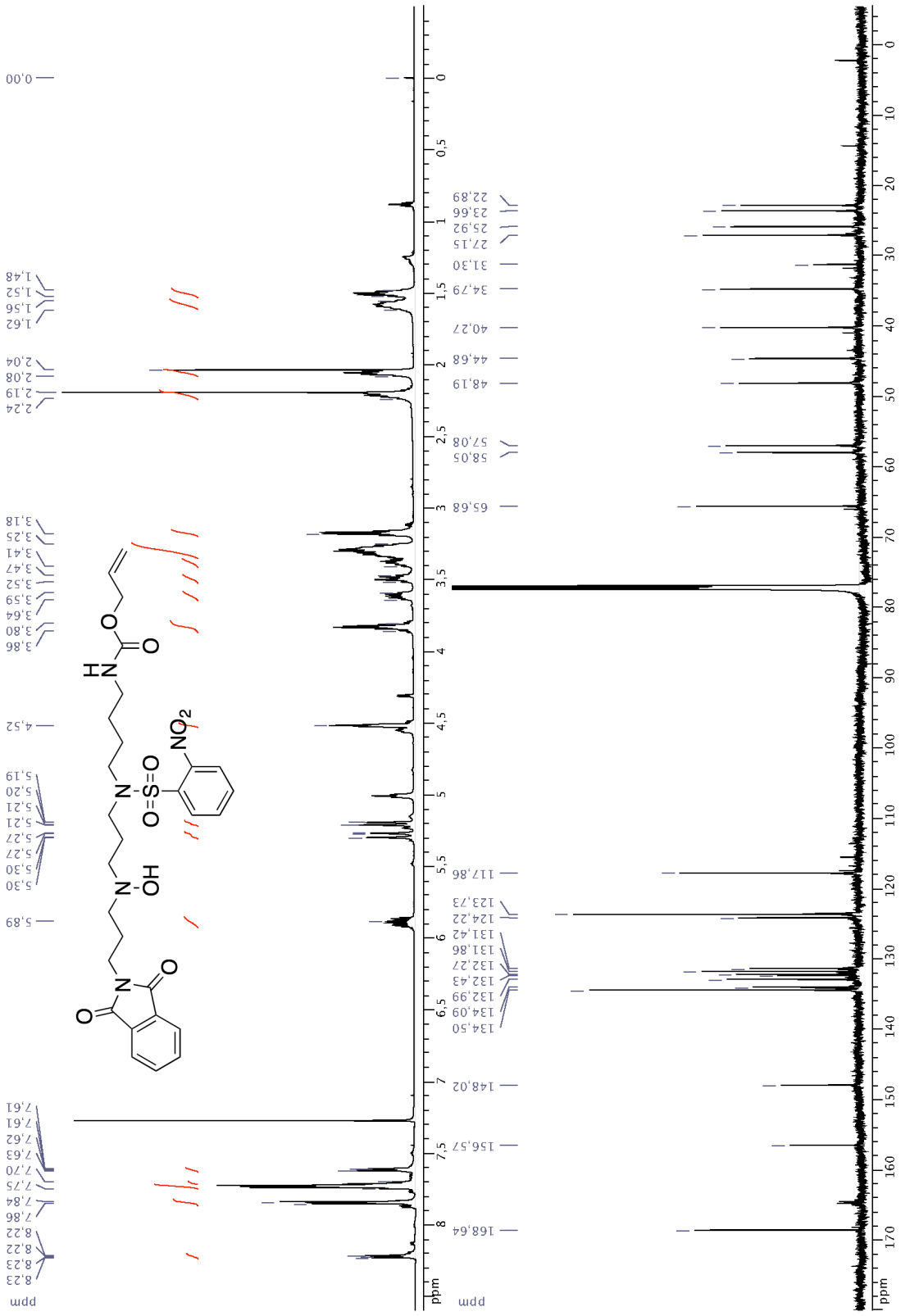
Tetraamine Derivative **53**



Tetraamine Derivative **54**



Tetraamine Derivative **55**



— Chapter 3 —

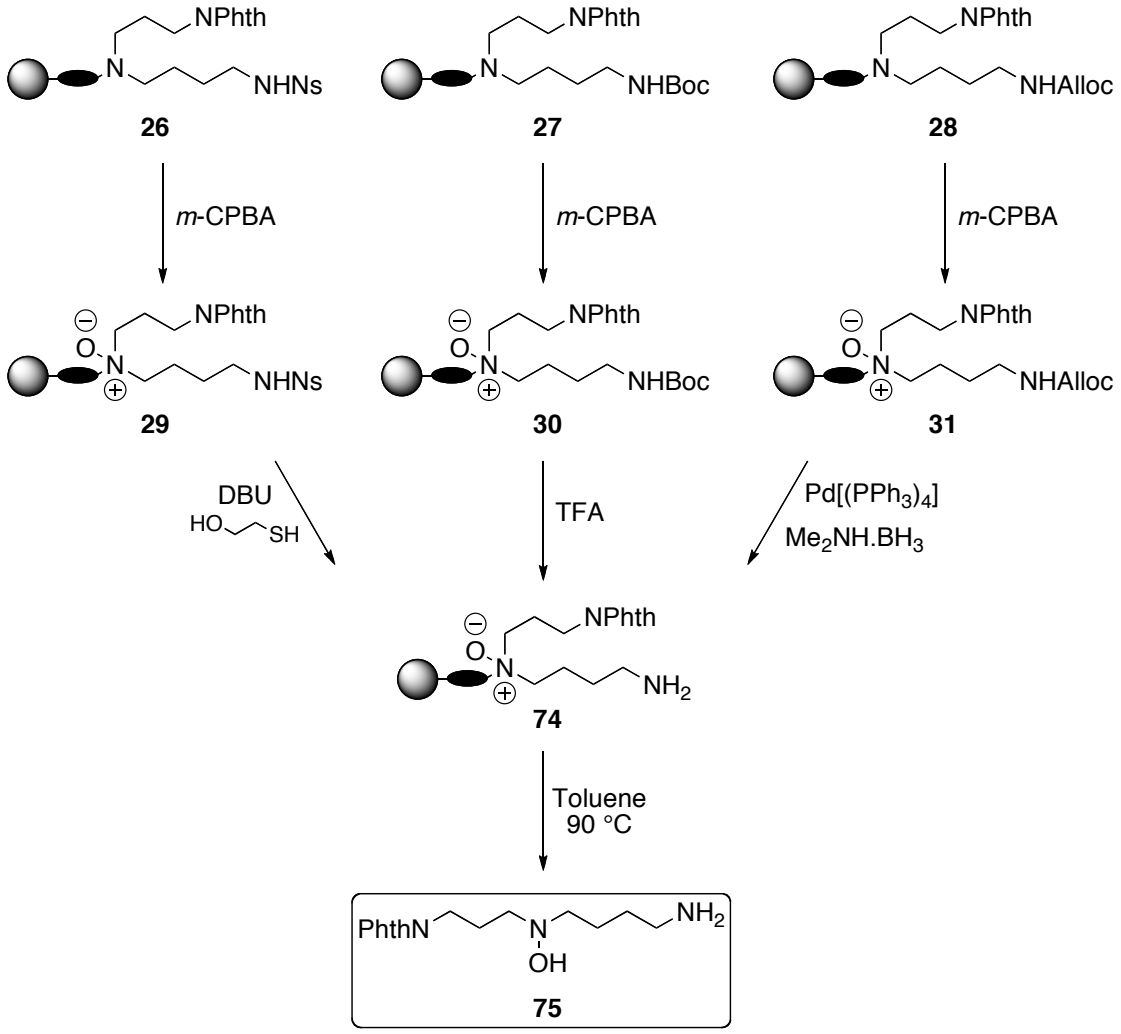
Selective Deprotections of *N*-Amine Oxide Resins

After the successful development of the method for the synthesis of any protected polyamine backbone, optimisation of the deprotection processes on solid-support was investigated. The question to be answered was whether it would be possible to remove the several protective groups selectively while retaining the control over the regioselectivity in the oxidation step. A straight-forward solution to the problem could be rather simple: namely if the deprotection procedure would be performed in-between the two steps of the oxidative cleavage protocol, i.e., after the oxidation of the homobenzylic N-atom and before the thermal *Cope* elimination, no selectivity problem would arise. First of all, however, the compatibility of the deprotection conditions and the amine oxide functionality on the resin had to be tested.

1. Selective Deprotections of Triamine Oxide Resins

We started our investigation of the selective removal of *N*-protective groups on *N*-amine oxide resins with the triamine resins **26–28**, which contain the Ns, Boc and Alloc protecting groups, respectively (Scheme 1). The three model resins were oxidised under the usual conditions with *m*-CPBA in CH_2Cl_2 to give the three amine oxide resins **29–31.** Resin **29** was then treated with 2-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF at 23 °C to afford primary amine resin **74**.[1] The presence of the primary amine functionality was confirmed by the *Kaiser* test.[2] Heating of the resin **74** up to 90 °C in toluene afforded finally monoprotected, mono-hydroxylated triamine **75** in 28% yield after purification by reverse phase HPLC. The same compounds **75** was also obtained by removal of the Boc and Alloc groups of **30**

and **31** using trifluoroacetic acid (TFA) in CH_2Cl_2 at 23 °C (29% yield) or [$Pd(PPh_3)_4$] and $Me_2NH{\bullet}BH_3$ in CH_2Cl_2 at 23 °C (26% yield), respectively.[3,4] The product obtained from resin **30** was initially accompanied by a trifluoroacetamide derivative, which was formed in the course of the removal of the Boc protective group. The respective reaction was already observed in peptide synthesis using TFA. The trifluoroacetamide derivative was no more detected while using a 2 M NaOH solution during the work-up in order to hydrolyse it.

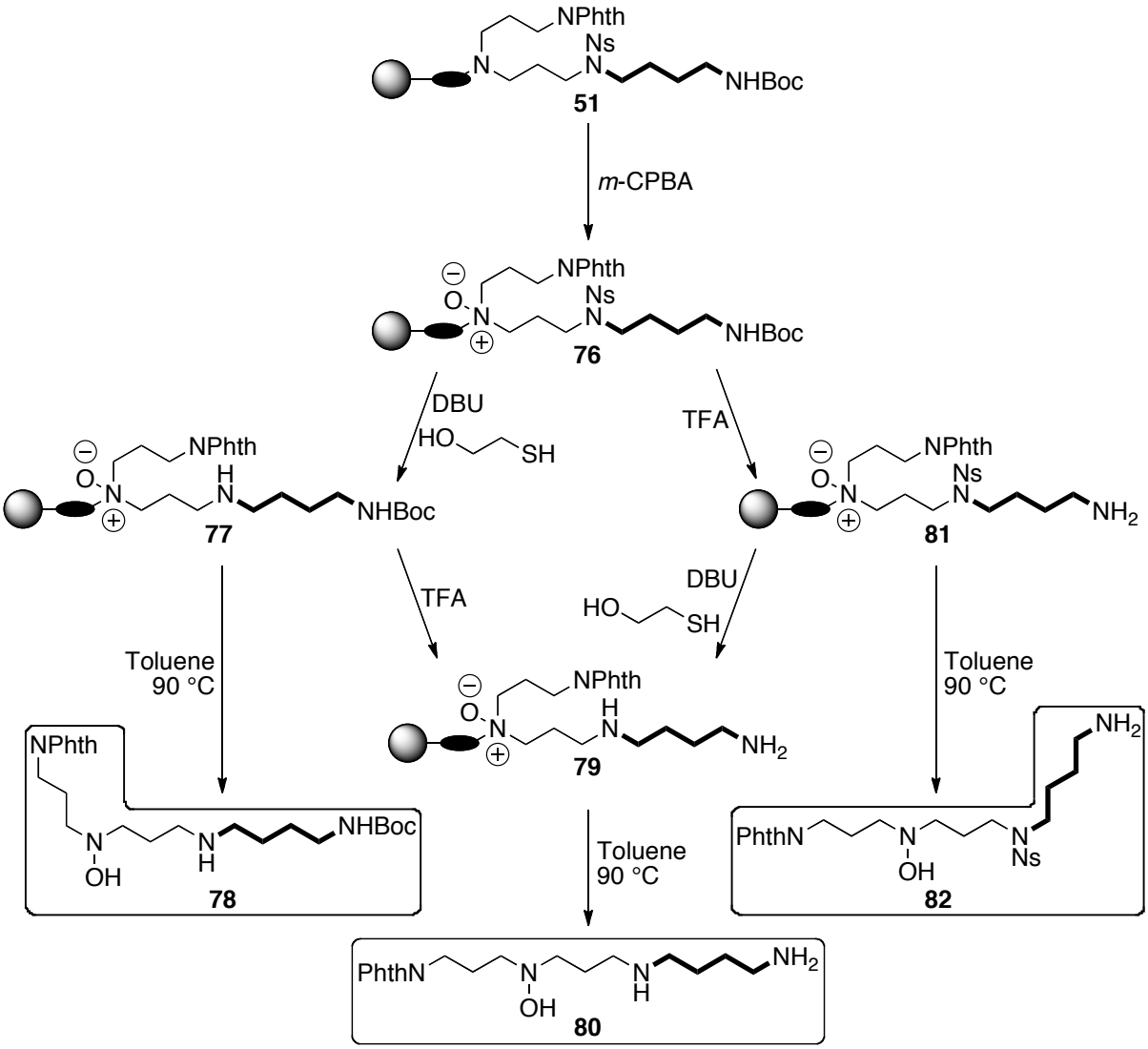


Scheme 1. Selective deprotection of triamine oxide resins.

2. Selective Deprotections of Tetraamine Oxide Resins

2.1. Orthogonality of Protective Groups

Tetraamine oxide resin **76** (resulting from oxidation of resin **51**), contains Phth, Ns and Boc groups and was chosen as a model compound to test the orthogonal removal of Ns and Boc from the *N*-oxide polyamine backbone. To this end, two parallel pathways were followed (Scheme 2).



Scheme 2. Orthogonality of Phth, Ns and Boc.

At first, the Ns moiety was removed by the action of 2-mercaptoethanol and DBU as described before, which delivered resin **77** and, after heating up to 90 °C, the *N*-hydroxylated tetraamine derivative **78**. ESI-MS of the resulting

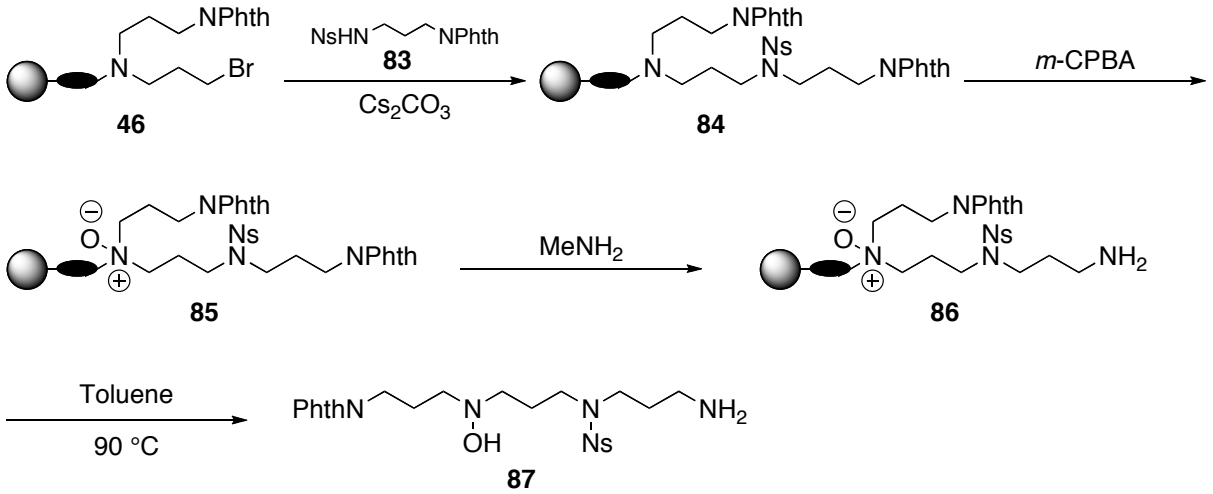
mixture showed that the amine oxide and the protected primary amine were not affected by these deprotection reaction conditions. Exposure of amine oxide resin **77** to TFA to remove the Boc group delivered resin **79**. Liberation of the Boc of the primary amine was proven by the *Kaiser* test.[2] Thermal treatment of resin **79** finally gave rise to the tetraamine derivative **80**, containing a primary and a secondary amine functionalities. On the second pathway, amine oxide resin **76** was submitted to the reversed succession of the deprotection reactions. Removal of the Boc group in the first step delivered resin **81**, which furnished the bis-protected tetraamine derivative **82** upon heating or, after the displacement of the Ns group, resin **79**, and finally again the *N*-hydroxylated tetraamine derivative **80**. On the stage of resin **81**, the presence of the primary amine was evidenced by the *Kaiser* test.[2] So far, derivatives **78**, **80** and **82** were not purified neither fully characterised by NMR since these compounds were not considered as final products but as intermediates for the preparation of fully unprotected *N*-hydroxylated penta-amines and natural products. The mixtures resulting of the cleavage of resin **77**, **79** and **81** were submitted to high resolution mass spectrometry (HR-MS) which gave a high accuracy mass of the desired tetraamine derivatives. We were confident that the deprotection steps leading to unprotected tetraamine **78**, **80** and **82** worked properly since no traces of intermediary products were detected. These results demontrate that the two pathways are comparable, meaning that the presence of a primary or a secondary amine has no influence on the subsequent deprotection reaction. Consequently the order of the different removals can be performed independently at the time of a total synthesis.

2.2. Selective Removal of the Phthaloyl Protective Group

In the previous examples, the Phth group was retained in the molecules since we wished to obtained final products that contained a chromophoric group. This simplified work-up and purification procedures, particularly the UV-detection of the compounds in HPLC. The removal of the Phth group, however, was also an issue, and an interesting phenomenom was observed

with this protective group. It was found that it was particularly hard to remove the Phth group from an N-atom that is merely linked to the resin through a propylene moiety.

This effect is particularly well illustrated by the behaviour of resin **84**, prepared from resin **46** by reaction with bis-protected diamine **83** (Scheme 3). Oxidation of resin **84** as usual with *m*-CPBA delivered resin **85**. To remove a Phth protective group, several methods are available. The most common, usually the method of choice, is hydrazinolysis[5], which, however, is not suited to our case. Hydrazinolysis requires temperatures of 80 °C or higher, which would initiate the *Cope* elimination and cleavage of the substrate from the resin. Transimidation by treatment with methylamine in NMP at 23 °C for 50 h,[6] was finally employed to prepare resin **86**, a method that was already used in our group before for the preparation of cinnamoyl polyamine derivatives.



Scheme 3. Phthaloyl selective deprotection.

Thermal treatment of resin **86** resulted in tetraamine derivative **87**, which was immediately submitted to ESI-MS for analysis (Figure 2). The MS showed a major signal at *m*/*z* 520, which corresponds to the $[M+H]^+$ quasi molecular ion of compound of **87** (or an isomer of it where the Phth is present at the other extremity of the molecule). Evidently, only one of the two Phth groups was removed by the deprotection reaction. Three minor compounds were also

detected. At *m*/*z* 390, a signal corresponding to the complete removal of the two phth groups (**88**). A peak at *m*/*z* 551, compatible with a compound where one Phth was released but where the other one was only « half-deprotected » (**89**) — addition of methylamine to the phthalimide moiety — and a signal at *m*/*z* 681, which was consistent with the « half deprotection » of only one Phth group (**90**). To determine the structure of **87**, CID of the ion detected at *m*/*z* 520 was undertaken (Figure 3).

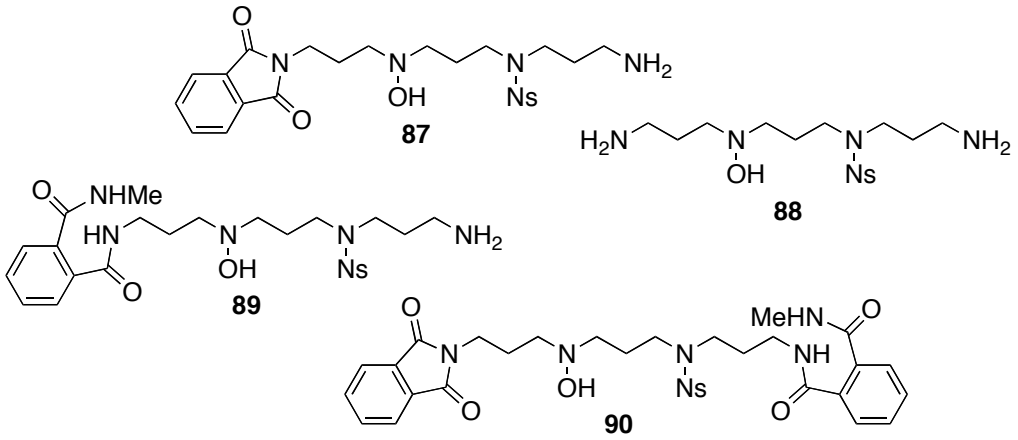


Figure 1. The products of the deprotection reaction.

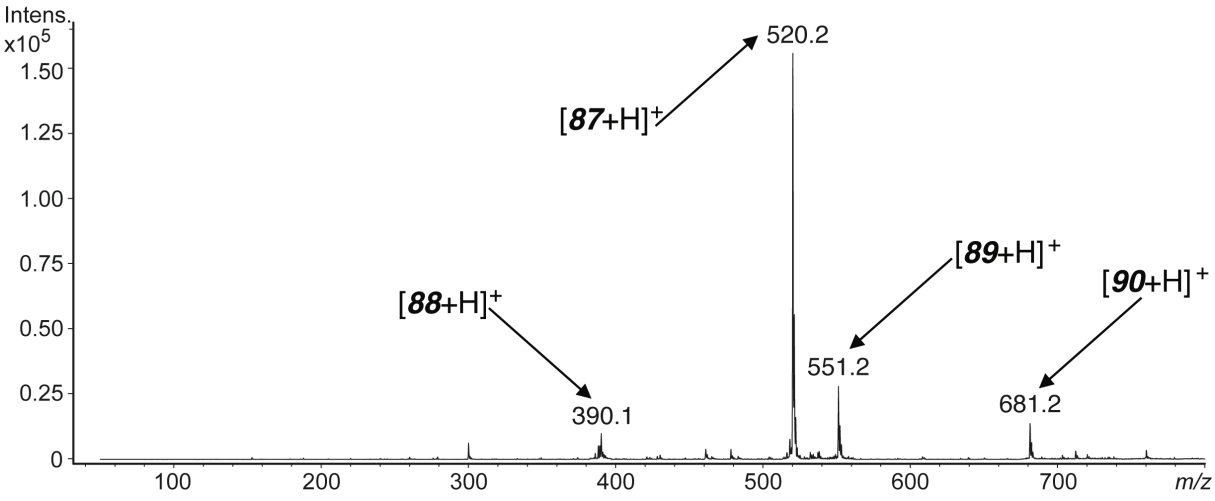


Figure 2. Crude **87** ESI-MS.

Three fragment ions were recognised (Figure 3). The mass peak at *m*/*z* 503 corresponds to the loss of ammonia from the resulting primary amine. The peak at *m*/*z* 188 is consistent with an *alpha* cleavage next to a N-atom including

the phthalimide moiety and three methylene groups. Unfortunately these two fragments cannot confirm the structure of **87** since these fragmentations could happened on the two possible mono deprotected products. The key fragment at *m*/*z* 300 (*alpha* cleavage next to a N-atom), indicates without any doubt that the displaced Phth protective group was the most far away from the polymer.

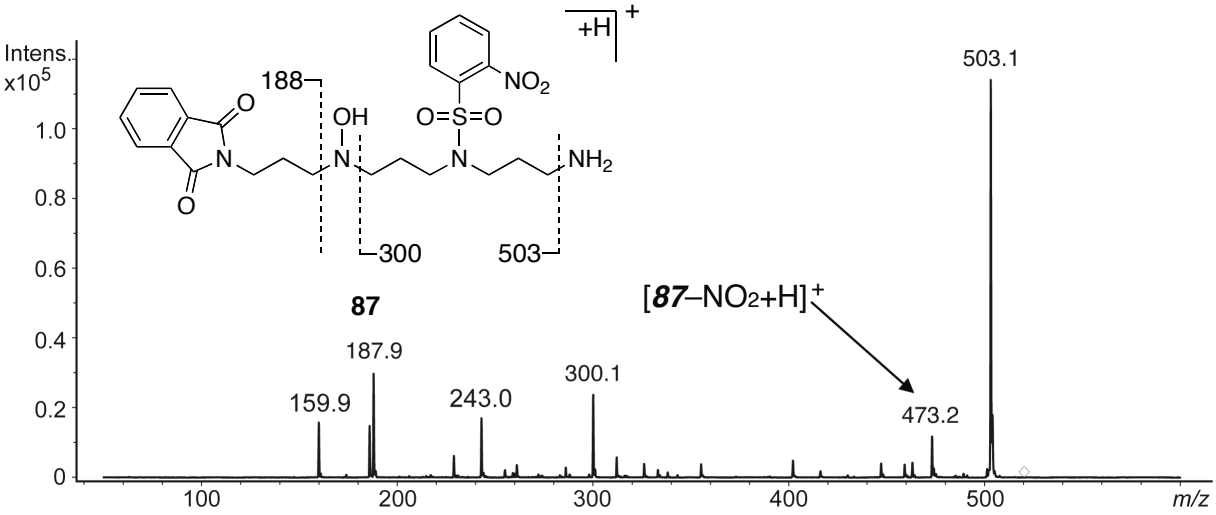


Figure 3. Confirmation of the structure of **87** by MS/MS of *m*/*z* 520.

3. CONCLUSIONS

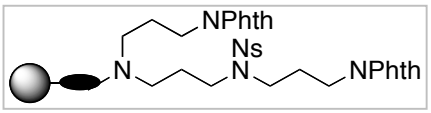
We have shown that Phth, Ns, Boc and Alloc protective groups can be removed from N-atom of solid-bound polyamines selectively in the presence of an amine oxide functionality and that the latter is not affected by the several deprotection conditions. This allows to believe that the efficient solid-phase synthesis of mono *N*-hydroxylated polyamine derivatives, thus, represents an efficient alternative to the « in-solution » methodologies for the synthesis of longer polyamine like penta- or hexaamine derivatives and especially for the preparation of natural products.

4. EXPERIMENTAL SECTION

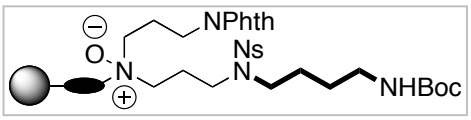
Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. Resin used: *Merrifield* polymer 200–400 mesh, 2% divinylbenzene, loading ~2.1 mmol Cl g^{-1} resin from *Fluka*. For the solid-phase reactions an *Advanced ChemTech PLS 6 Organic synthesiser* was used. IR spectra were recorded on a *Perkin–Elmer* 1600 Series FT-IR spectrophotometer and for the final products, an *OMNILAB* FT/IR 4100 spectrophotometer. *N*-Hydroxypolyamine derivatives were purified by preparative HPLC; chromatograms were recorded with *Dynamax* solvent delivery system model SD-300 coupled with a *Dynamax* absorbance detector model UV-1; column used: *Kromasil* KR100-10C18. Final products ^{1}H-NMR spectra in D_2O were measured with a *Bruker* AV-600 (600 MHz); δ rel. to TSP (δ 0.00 ppm). Final products ^{13}C-NMR spectra in D_2O were measured with a *Bruker* AV-600 (150 MHz); δ rel. to TSP (δ 1.7 ppm); multiplicities from DEPT-135 and DEPT-90 experiments; the assignments of the carbon resonances followed from HSQC experiments. ESI-MS was performed on a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH*, Bremen, Germany), equipped with a combined *Hewlett-Packard* Atmospheric Pressure Ion (API) source (*Hewlett–Packard Co.*, Palo Alto, CA, USA). HR-MS: High-resolution electrospray mass spectra were recorded on a *Bruker* maXis QTOF-MS instrument (*Bruker Daltonics GmbH*, Bremen, Germany). The samples were dissolved in MeOH and analyzed *via* continuous flow injection at 3 μL/min. The mass spectrometer was operated in positive ion mode with a capillary voltage of 4 kV, an endplate offset of 500 V, nebulizer pressure of 5.8 psig, and a drying gas flow rate of 4 L/min at 180 °C. The instrument was calibrated with a Fluka electrospray calibration solution (Sigma-Aldrich, Buchs, Switzerland) that has been 100 times diluted with acetonitrile. The resolution was optimized at 30'000 FWHM in the active focus mode. The accuracy was better than 2 ppm in a mass range between *m*/*z* 118 and 2721. All solvent used were purchased in best LC-MS qualities. Proof of structure and purity of the final polyamine derivatives was provided by NMR spectra and MS/MS.

Elemental analyses were not appropriate for the polyamine derivatives since the compounds arose as waxy or glassy solids only, from which the last solvent residues can hardly be removed.

4.1. Synthesis of Solid-Supported Tetraamine Oxide Derivatives

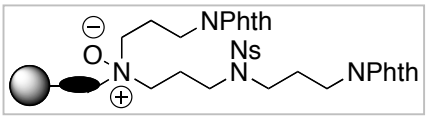
Alkylation of Resin 46 with *N*-[3-(2-Nitrophenylsulfonamido)propyl]phthalimide (83) to Form Resin 84. 

Resin **46** (0.3 mmol) was swelled in DMF (10 mL) at 50 °C for 15 min. Cs_2CO_3 (0.49 g, 1.5 mmol) and *N*-[3-(2-nitrophenylsulfonamido)propyl]phthalimide (**83**, 0.58 g, 1.5 mmol) were added, and the suspension was agitated at 50 °C for 24 h. Resin **84** was filtered off, washed successively with DMF, NMP/H_2O (1:1), NMP, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1770 (CO), 1709 (CO), 1509 (NO_2), 1363 (SO_2), 1339 (NO_2), 1163 (SO_2) cm^{-1}.

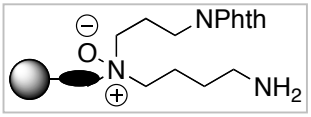
Oxidation of Resin 51 with *m*-CPBA to Give Resin 76 (General Procedure). 

Resin (obtained from 0.3 mmol of resin **14**) was swelled in CH_2Cl_2 (5 mL) for 15 min. *m*-CPBA (0.26 g, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added, and the suspension was agitated for 3 h at 23 °C. Resin **76** was filtered off, washed successively with DMF, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1770 (CO of Phth), 1712 (CO of Boc/Phth), 1509 (NO_2), 1452 (SO_2), 1363 (NO_2), 1165 (SO_2) cm^{-1}.

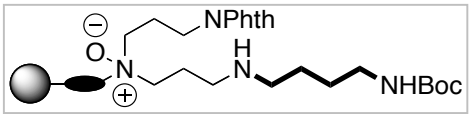
Oxidation of Resin 84 with *m*-CPBA to Give Resin 85. According to the general procedure, resin **84** was oxidised with *m*-CPBA to give resin **85**. IR ν = 1770 (CO), 1712 (CO), 1509 (NO_2), 1369 (SO_2), 1339 (NO_2), 1165 (SO_2) cm^{-1}.



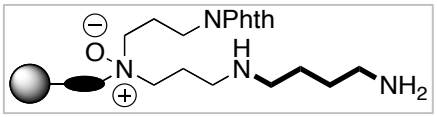
4.2. Removal of Protective Groups

Removal of the Ns Protective Group of Resin 29 to Give Resin 74 (General Procedure). 

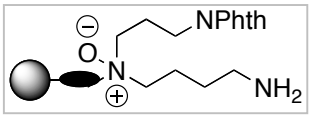
Resin **29** (0.5 mmol) was swelled in DMF (3 mL) for 15 min. DBU (1.49 mL, 9.8 mmol) and 2-mercaptoethanol (0.35 mL, 5.0 mmol) were added. The suspension was agitated for 2.5 h at 23 °C. Resin **74** was filtered off and washed successively with DMF (3×), NMP (3×), CH_2Cl_2 (3×), MeOH, CH_2Cl_2 and MeOH and dried *in vacuo*. The *Kaiser* test[2] was performed to prove the presence of primary amine group. IR ν = 1770 (CO), 1707 (CO) cm^{-1}.

Removal of the Ns Protective Group of Resin 76 to Give Resin 77. 

According to the general procedure, Ns group of resin **76** (0.3 mmol) was removed to give resin **77**. IR ν = 1770 (CO of Phth), 1712 (CO of Boc/Phth) cm^{-1}.

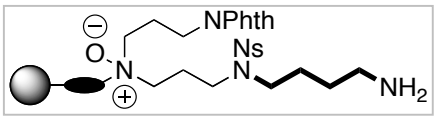
Removal of the Ns Protective Group of Resin 81 to Give Resin 79. 

According to the general procedure, Ns group of resin **81** (0.3 mmol) was removed to give resin **79**. IR ν = 1770 (CO), 1707 (CO) cm^{-1}.

Removal of the Boc Protective Group of Resin 30 to Give Resin 74 (General Procedure). 

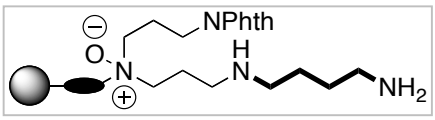
Resin **30** (0.3 mmol) was swelled in CH_2Cl_2 (3 mL) for 15 min. TFA (0.46 mL, 6.0 mmol) was added and the suspension was agitated for 12 h at 23 °C. Resin **74** was filtered off and washed successively with CH_2Cl_2, CH_2Cl_2/DIEA (1:1), H_2O, 2 M NaOH, H_2O, CH_2Cl_2, and MeOH and dried *in vacuo*. The *Kaiser* test[2] was performed to prove the presence of primary amine group. IR ν = 1770 (CO), 1707 (CO) cm^{-1}.

Removal of the Boc Protective Group of Resin 76 to Give Resin 81.



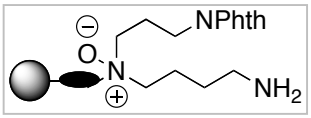
According to the general procedure, Boc group of resin **76** (0.3 mmol) was removed to give resin **81**. IR ν = 1770 (CO), 1707 (CO), 1509 (NO_2), 1452 (SO_2), 1369 (NO_2), 1165 (SO_2) cm^{-1}.

Removal of the Boc Protective Group of Resin 77 to Give Resin 79.



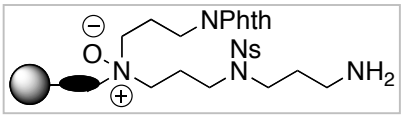
According to the general procedure, Boc group of resin **77** (0.3 mmol) was removed to give resin **79**. IR ν = 1770 (CO), 1707 (CO) cm^{-1}.

Removal of the Alloc Protective Group of Resin 31 to Form Resin 74. Resin



31 (0.3 mmol) was swelled in CH_2Cl_2 (3 mL) for 15 min. [$Pd(PPh_3)_4$] (34 mg, 0.03 mmol) and $Me_2NH.BH_3$ (0.71 g, 12.0 mmol) was added and the suspension was agitated for 1.5 h at 23 °C under Ar atmosphere. Resin **74** was filtered off and washed successively with CH_2Cl_2, 0.2% TFA in CH_2Cl_2, CH_2Cl_2, DIEA/DMF (1:1), dioxane/H_2O (9:1), MeOH, DMF and CH_2Cl_2 and dried *in vacuo*. The *Kaiser* test[2] was performed to prove the presence of primary amine group. IR ν = 1770 (CO), 1707 (CO) cm^{-1}.

Removal of the Phth Protective Group[*] of Resin 85 to Form Resin 86. Resin



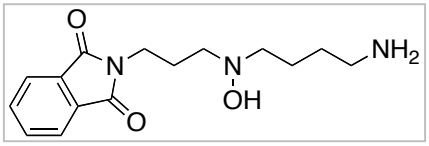
85 (0.1 mmol) was swelled in NMP (2 mL). Methylamine (41% in H_2O, 75 μL, 1.00 mmol) was added. The suspension was agitated at 23 °C for 50 h. Resin **86** was filtered off and washed successively with NMP (2×), NPM/H_2O (1:1), NMP, DMF, CH_2Cl_2 and MeOH and dried *in vacuo*. The *Kaiser* test[2] was performed to prove the presence of primary amine group IR ν = 1770 (CO), 1707 (CO), 1509 (NO_2), 1452 (SO_2), 1369 (NO_2), 1165 (SO_2) cm^{-1}.

[*] For optimisation of reaction conditions, see 3. in Chapter 4.

4.3. Liberation of the Tri- and Tetraamine Derivatives

***Cope* Elimination (General Procedure):** The resin (obtained from 0.3 mmol of resin **14**) was swelled in toluene (10 mL). The suspension was agitated for 2 h at 90 °C. The resin was filtered off and washed with toluene, CH_2Cl_2 and MeOH. The combined filtrates were evaporated to give a yellow oil, which was purified by HPLC as described below.

***N*-(8-Amino-4-hydroxy-4-azaoctyl)phthalimide (75) from Resin 74.** HPLC



(H_2O/MeCN/TFA 85:15:0.1, 25 mL min^{-1}, λ = 280 nm) gave **75** (as a trifluoroacetic acid salt) as a colourless solid (26–29% from resin **14** depending on the deprotection conditions used, see 1.).

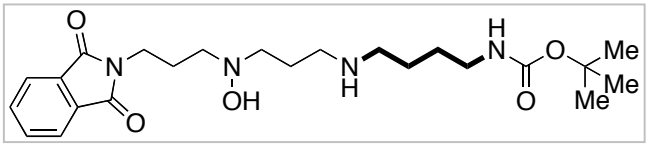
IR ν = 1770 (CO),1707 (CO) cm^{-1}.

^{1}H-NMR (600 MHz, D_2O, 278 K) δ = 7.65–7.62 (m, 2 H, 2 arom. CCH of Phth), 7.59–7.56 (m, 2 H, 2 arom. CCHC*H* of Phth), 3.57 (t, $^3J_{H,H}$ = 6.7 Hz, 2 H, PhthNCH_2), 3.27–3.07 (m, 4 H, CH_2NOHCH_2), 2.78 (t, $^3J_{H,H}$ = 7.6 Hz, 2 H, NH_2CH_2), 2.02–1.86 (m, 2 H, PhthNCH_2CH_2), 1.72–1.54 (m, 2 H, NH_2CH_2-CH_2CH_2), 1.54–1.47 (m, 2 H, NH_2CH_2CH_2) ppm.

^{13}C-NMR (150 MHz, D_2O, 278 K) δ = 170.3 (s, 2 CO of Phth), 134.3 (d, 2 arom. CCHCH of Phth), 130.9 (s, 2 arom. C of Phth), 122.9 (d, 2 arom. CCH of Phth), 58.0, 56.3 (2 t, CH_2NOHCH_2), 38.4 (t, NH_2CH_2), 34.3 (t, PhthNCH_2), 23.4 (t, $NH_2CH_2CH_2$), 22.6 (t, PhthNCH_2CH_2), 20.2 (t, $NH_2CH_2CH_2CH_2$) ppm.

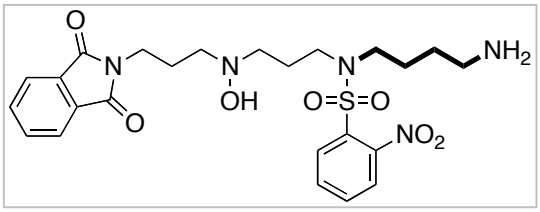
ESI-MS m/z = 292.1 (100, [M+H]$^+$).

***tert*-Butyl *N*-(5-Amino-9-hydroxy-12-phthalimido-5,9-diazadodecyl)carbamate (78) from Resin 77.**



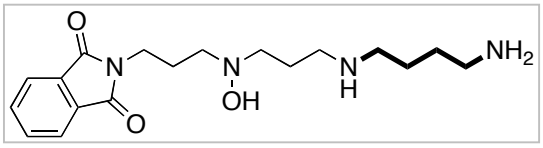
HR-MS: calcd. for $C_{23}H_{37}N_4O_5$ 449.27585; found 449.27578.

***N*-[12-Amino-4-hydroxy-8-(2-nitrophenylsulfonamido)-4,8-diazadodecyl]-phthalimide (82) from Resin 81.**



HR-MS: calcd. for $C_{24}H_{32}N_5O_7S$ 534.20170; found 534.20133.

***N*-(8,12-Amino-4-hydroxy-4,8-azadodecyl)phthalimide (80) from Resin 79.**



HR-MS: calcd. for $C_{18}H_{29}N_4O_3$ 349.22342; found 349.22366.

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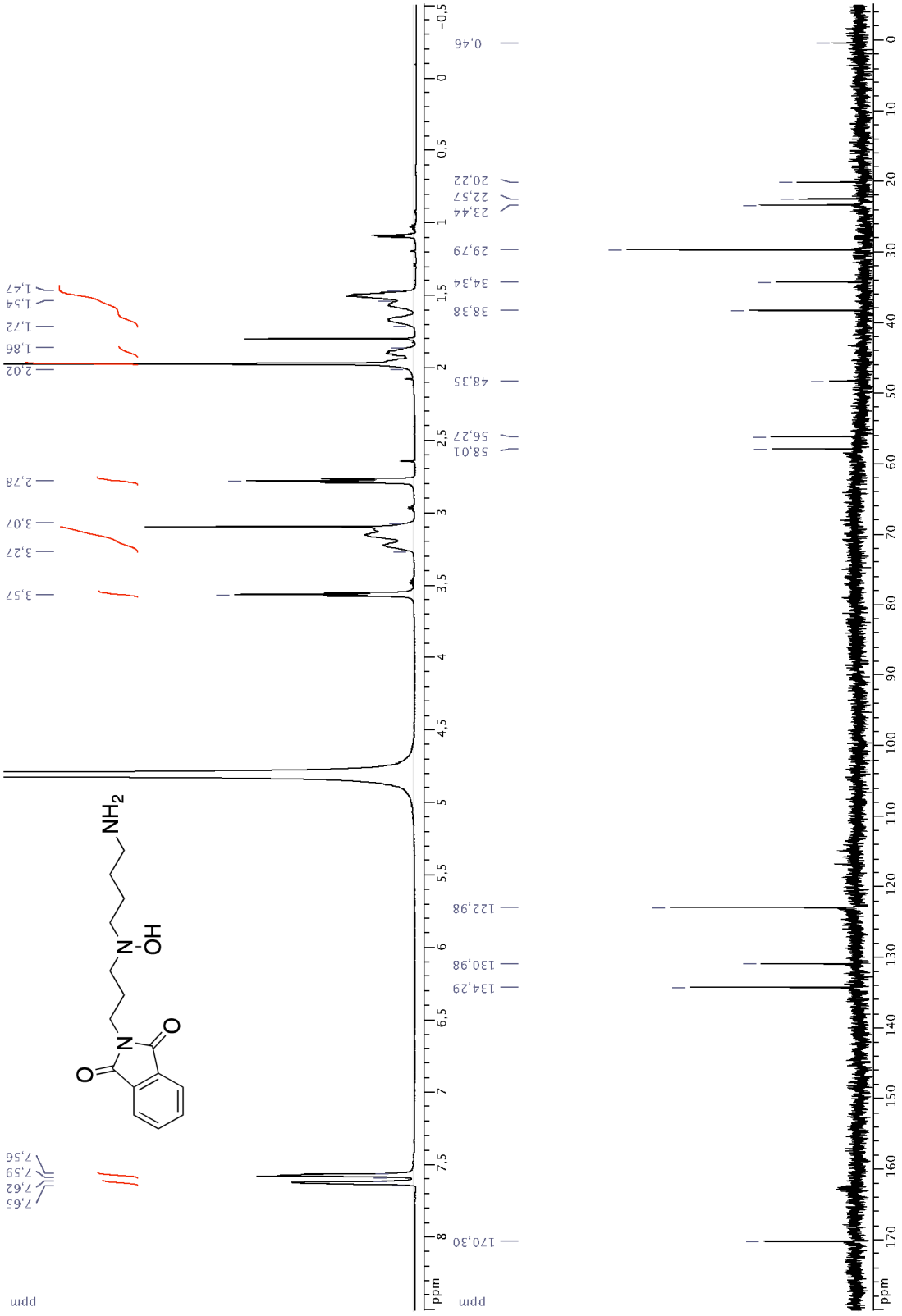
(6) Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, *20*, 2093.

APPENDICE — NMR OF FINAL COMPOUND

Triamine Derivative **75**



— Chapter 4 —

Applications to the Preparation of Penta- and Hexaamine Derivatives

A flexible method for the synthesis of any linear protected and unprotected hydroxypolyamines on solid-support has been developed.[1] Starting from "the centre" and using a protective group strategy, the backbone is constructed on solid-support, and, finally, the *Cope* elimination is used as the cleavage reaction, to introduce the desired *N*-hydroxy functionality at the end of the synthesis. This method was applied to the synthesis of tri- and tetraamine derivatives. The goal was then to prepare *N*-hydroxylated natural products found in spider venom. To our knowledge, no *N*-hydroxylated tetraamine derivatives were detected in spider venom. On the other hand, *N*-hydroxylated penta- and hexaamine derivatives were detected in *Agelenopsis aperta*'s venom (Figure 1).[2]

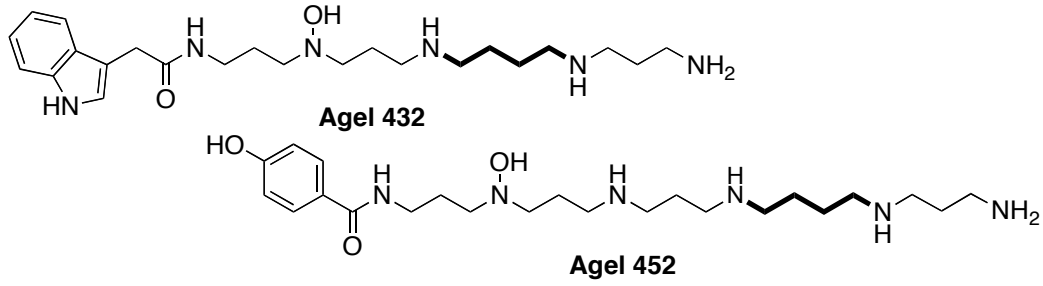
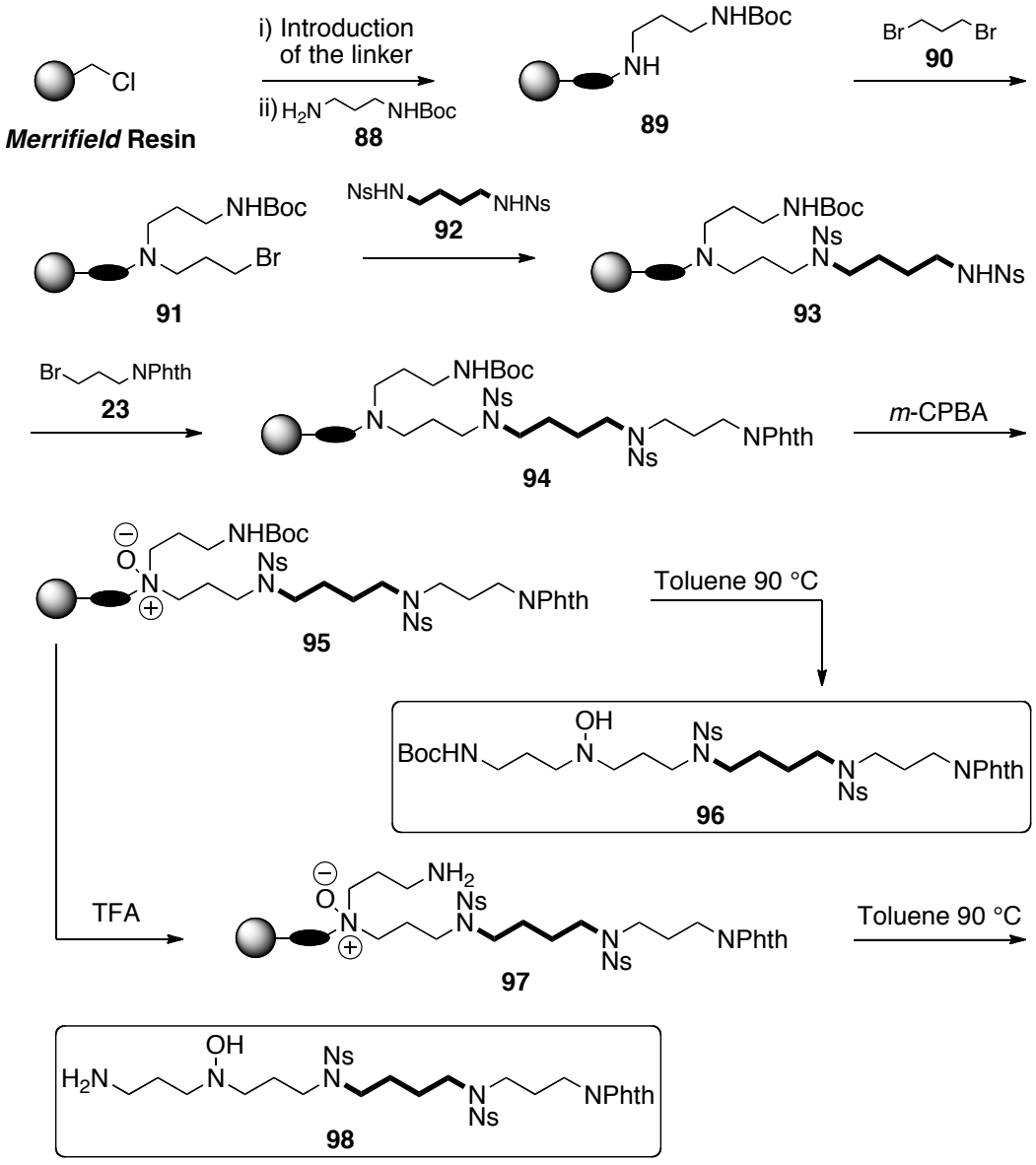


Figure 1. *N*-Hydroxylated pentaamine derivatives from *Agelenopsis aperta*'s venom.

1. Synthesis of Pentaamine Derivative

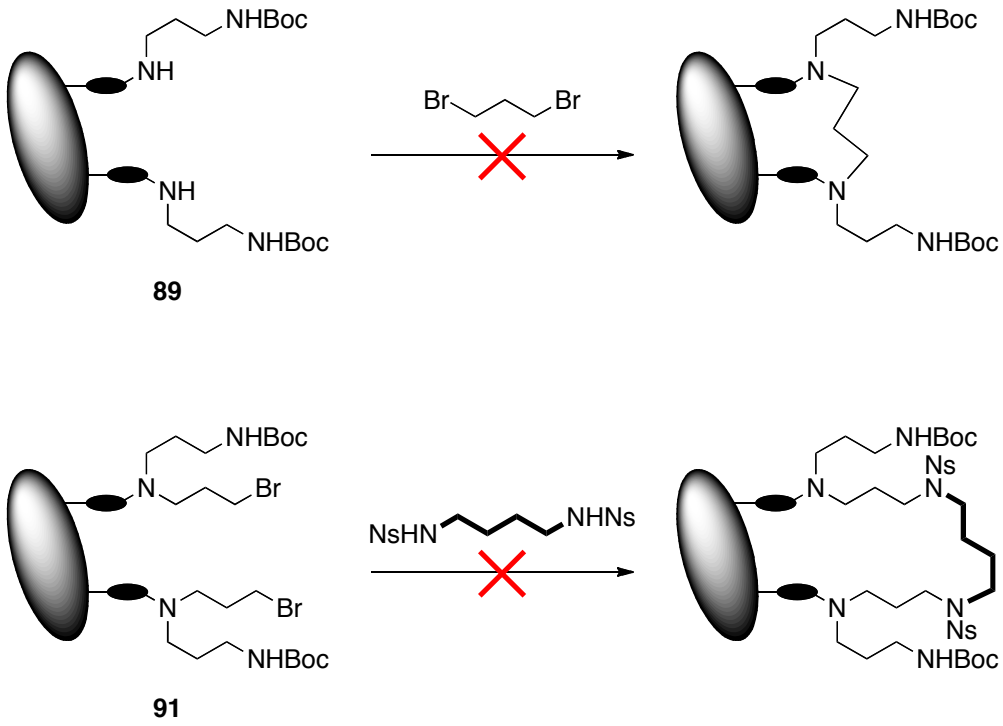
At the time of the synthesis of the tri and tetraamine derivatives we figured out that bromide–hydroxyl exchange[3-6] and the removal of a Phth protecting group at a terminal amine next to the resin are obviously not the most convenient transformations to perform on solid-phase. In order to overcome these tedious reactions and to take the opportunity to save one step, we

decided to make few changes in the synthesis of the polyamine backbone. The pentaamine derivative preparation begins, as already described previously (see Chapter 2), by the conversion of the *Merrifield* resin into resin **89** by introduction of the phenethyl bromide linker[6] followed by the coupling with *tert*-butyl *N*-(3-aminopropyl)carbamate (**88**) through nucleophilic substitution (Scheme 1). Since it was demonstrated that removal of a Phth protecting group at a terminal amine position nearby the resin shows some difficulties (Chapter 3), we decided to use a Boc group instead. The Boc group will be displaced in order to attach the desired chromophore later in the synthesis.



Scheme 1. Solid-support preparation of the pentaamine backbone.

The elongation of the polyamine backbone was subsequently effected by treatment of resin **89** with 1,3-dibromopropane (**90**) in NMP in presence of DIEA at 50 °C for 24 h. This alkylation step was crucial for the rest of the synthetic pathway. The conditions for the elongation of the backbone at the secondary amine position were already optimised for reactions with amino-bromide derivatives but not for the use of a symmetric dibromo compound like **90** because of its prominent ability to perform cross-linking within the solid-support (Scheme 2). ESI-MS measurements following the oxidative cleavage of resin **91** did not show any traces of cross-linked compound. In order to prepare pentaamine derivatives applying the elongation method by nucleophilic substitution, the bromo resin **91** was further extended by alkylation with *N*,*N*-bis-(2-nitrophenylsulfonyl)-1,4-diaminobutane (**92**) in presence of Cs_2CO_3 to form resin **93**. Although conditions of the reaction were already optimised and as a consequence perfectly under control (see Chapter 2), this step presented the same potential problem of cross-linking, and probably in a higher manner because of the higher flexibility of the arm. To our satisfaction, ESI-MS measurements of the mixture resulting of the oxidative cleavage of the resin **93** did not show any traces of cross-linked component.



Scheme 2. Cross-linking within the resin.

To complete the elaboration of the pentaamine backbone, a third alkylation by substitution reaction was performed with *N*-(3-bromopropyl)phthalimide **23** and Cs_2CO_3 to give **94,** which we submitted to the oxidation reaction with *m*-CPBA. An aliquot of resin **95** was cleaved yielding to the *N*-hydroxylated pentaamine derivative **96** to confirm that the last elongation and the oxidation steps occurred properly. Derivative **96** was neither purified nor fully characterised by NMR, since this compound was not considered as a final product. The mixture resulting of the cleavage of resin **95** was submitted to high resolution mass spectrometry (HR-MS) which gave a high accuracy mass of the desired pentaamine derivative **96**. We were confident that the entire synthetic pathway leading to **96** worked properly because no traces of intermediary products were detected. In order to prepare spider toxins the Boc protective group had to be released for further acylation of the position with the correct chromophores. The removal of the Boc was performed by using TFA in CH_2Cl_2 at 23 °C (see Chapter 3) to give resin **97**. The presence of the primary amine was confirmed by the *Kaiser* test.[7] Submission of an aliquot of resin **97** to the *Cope* elimination gave rise to pentaamine derivative **98**. Derivative **98** was neither purified, nor characterised by NMR since resin **97** was considered as an intermediate for further investigations. Nevertheless HR-MS confirmed that the deprotection of the Boc group worked fine since **96** was not observed during the measurements.

2. Stability of the Amine Oxide Derivatives

In the course of our investigations of spider venom, we recently highlighted the observation of partial reduction of *N*-hydroxylated compounds into the corresponding secondary amine derivative during APCI-MS measurements.[8] With the help of model compounds synthesised on solid-phase it was demonstrated that the reduction was pH- and concentration-dependent (see Chapter 5). It was then possible either to force the reduction by addition of a strong acid, namely TFA, or to inhibit it by addition of ammonia. From a synthetic point of view, the decomposition of the desired *N*-hydroxypolyamine into the polyamine was observed too. ESI-MS measurements of the

crude mixture resulting of the cleavage step showed, in some particular cases, the presence of the reduced product. In the case of the pentaamine derivative **96** (highly activated by the presence of the two Ns groups), firstly only the decomposition product was detected (Figure 2, A). Since the reduction of *N*-hydroxylamine was never observed during ESI-MS experiments, the reduced compound can only be a product of decomposition during the cleavage step. The cleavage procedure of amine oxide resin **95** was performed again but in presence of Et_3N to create basic conditions. ESI-MS measurements of the resulting mixture did not show any traces of decomposition product (Figure 2, B) and corroborated the observations of the MS investigations.

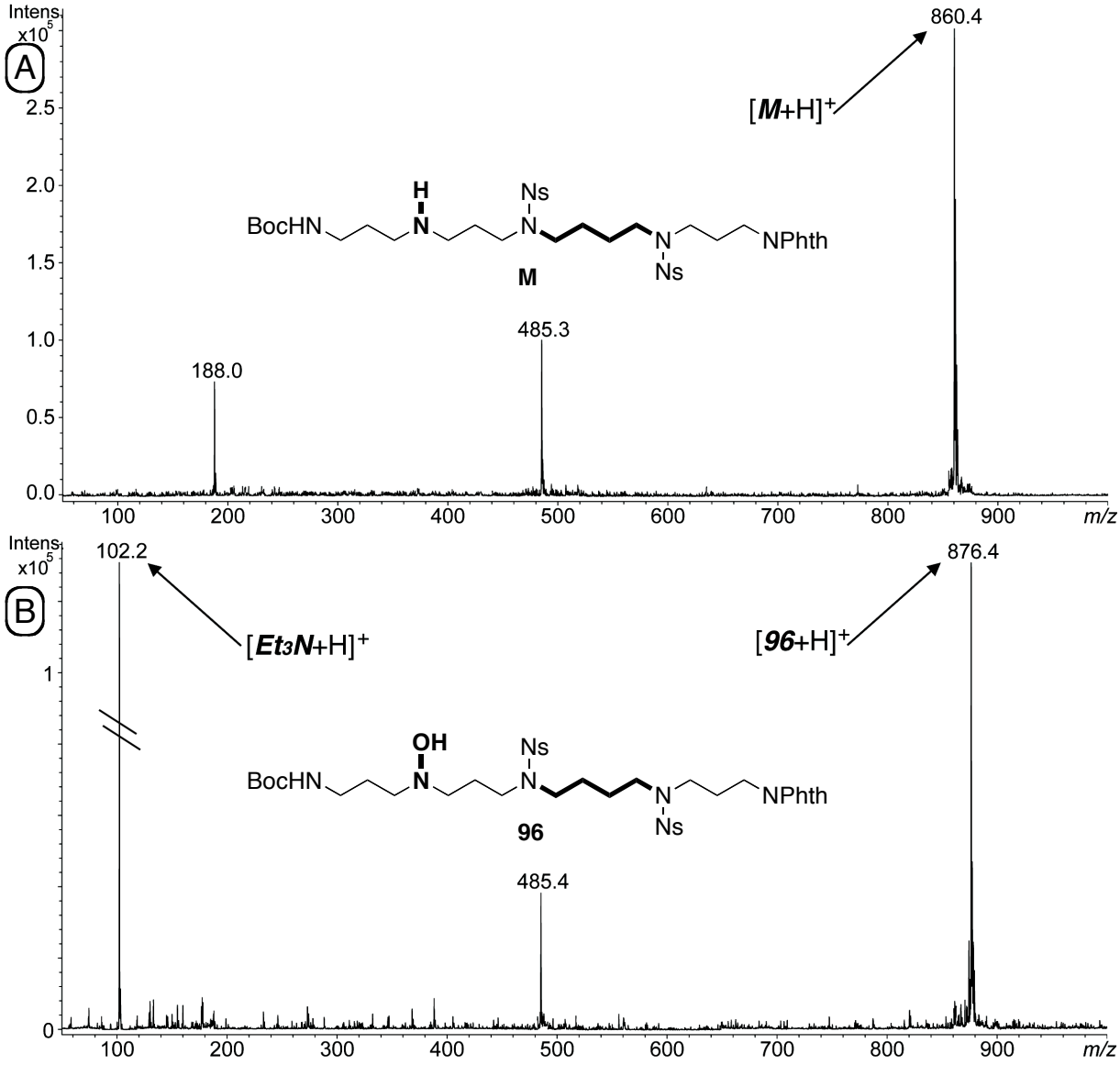


Figure 2. ESI-MS of crude pentaamine derivative **96**. A) Cleavage in absence of Et_3N. B) Cleavage in presence of Et_3N.

3. Attempts for the Preparation of Spider Toxin Natural Products

Four chromophores were detected in *Agelenopsis aperta* toxins.[2] In the following, one of them was chosen for the first attempt to prepare a natural product. Using 4-hydroxybenzoic acid (4-OH-Bz), **Agel 395** was defined as the target structure (Figure 3).

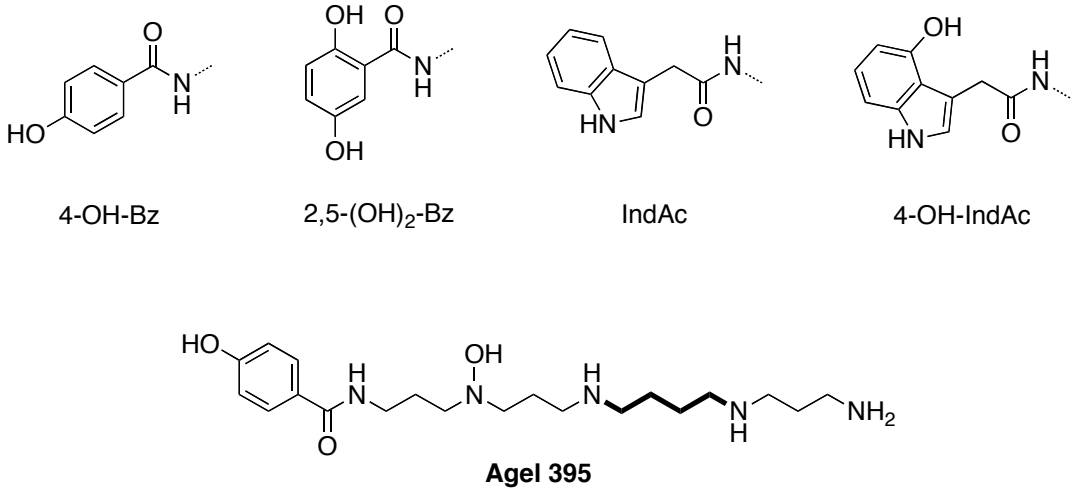
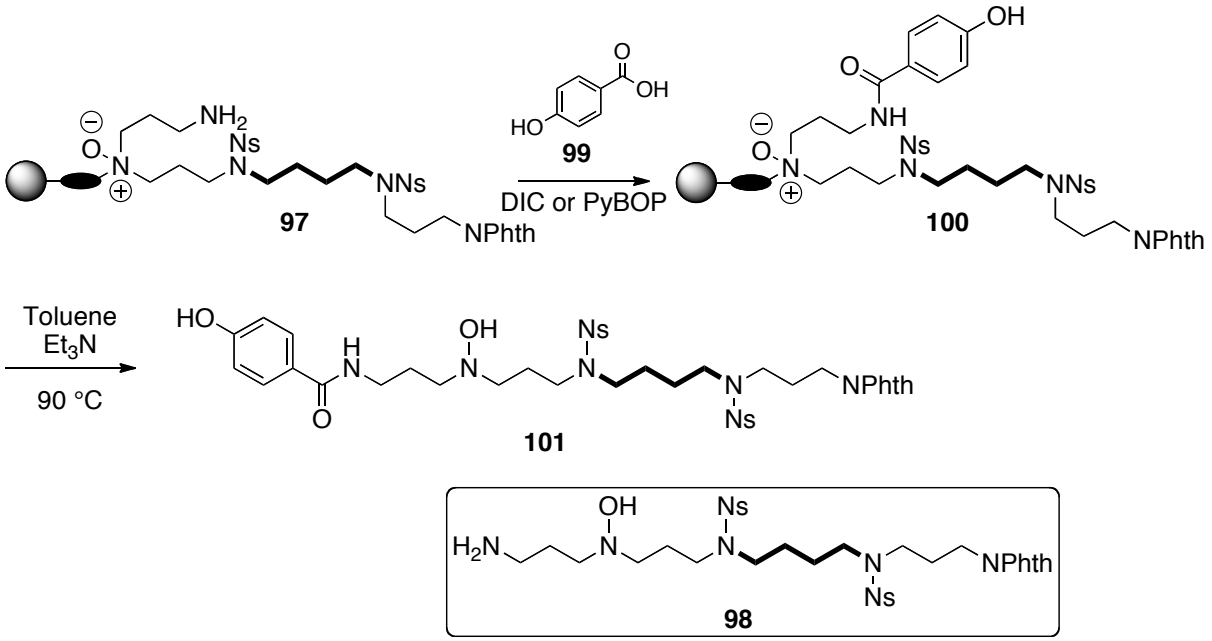


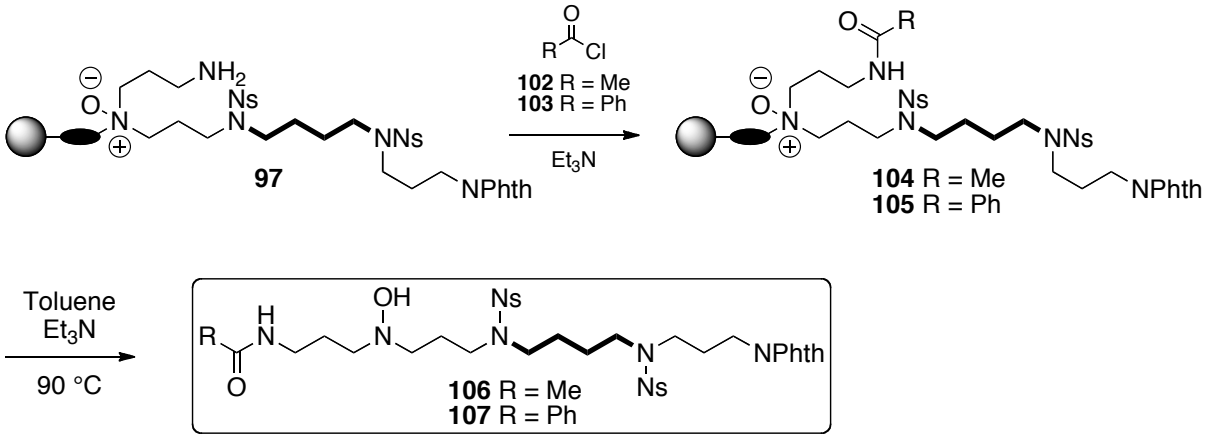
Figure 3. Lipophilic heads found in Agelenidae toxins and **Agel 395**.

In order to avoid additional steps with protection/deprotection of the chromophores (sensitive to oxidation conditions), the pentaamine oxide resin **95** was used and first selectively released of the Boc protective group by treatment with TFA to form the primary amine **97**[9] (confirmed with the *Kaiser* test[7]) (Scheme 1). To access the target molecule, the terminal primary amine has to be acylated with the carboxylic acid derivative **99** (Scheme 3). The procedure, already performed by our group to prepare acylpolyamine spider toxins using diisopropylcarbodiimide (DIC), was carried out with 4-hydroxybenzoic acid.[10] Unfortunately *Cope* elimination on resin **100** did not furnish the desired acylpolyamine derivative **101** but the non-acylated primary amine derivative **98**. The acylation reaction temperature was changed from 23 °C to 50 °C for 30 h, but again no traces of **101** were detected.



Scheme 3. Acylation attempts using 4-hydroxybenzoic acid in presence of DIC.

Attempts using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) at 23 and 50 °C were performed, but once more, only primary amine **98** was obtained. At this point we were wondering wether the proximity of the resin to the primary amine to be acylated would again play a role. In other words if the intermediary activated carboxylic acid is too massive to access the primary amine or if this position is not accessible enough to be acylated at all. We then decided to try to acylate resin **97** with the smallest acyl chloride, acetyl chloride (**102**) (Scheme 4). The reaction was run in CH_2Cl_2 in presence of Et_3N for 3 h at 23 °C.[11]



Scheme 4. Acylation by reaction with acyl chloride derivatives.

ESI-MS of crude **106** performed after the cleavage procedure showed that the acylation worked, but not completely (presence of primary amine **98**) (Figure 4). The correct structure of **106** was confirmed by MS/MS (Figure 5), Showing the acyl moiety fragmentation (*m*/*z* 776) followed by the loss of ammonia (*m*/*z* 758) and the confirmation of the polyamine backbone (*m*/*z* 719).

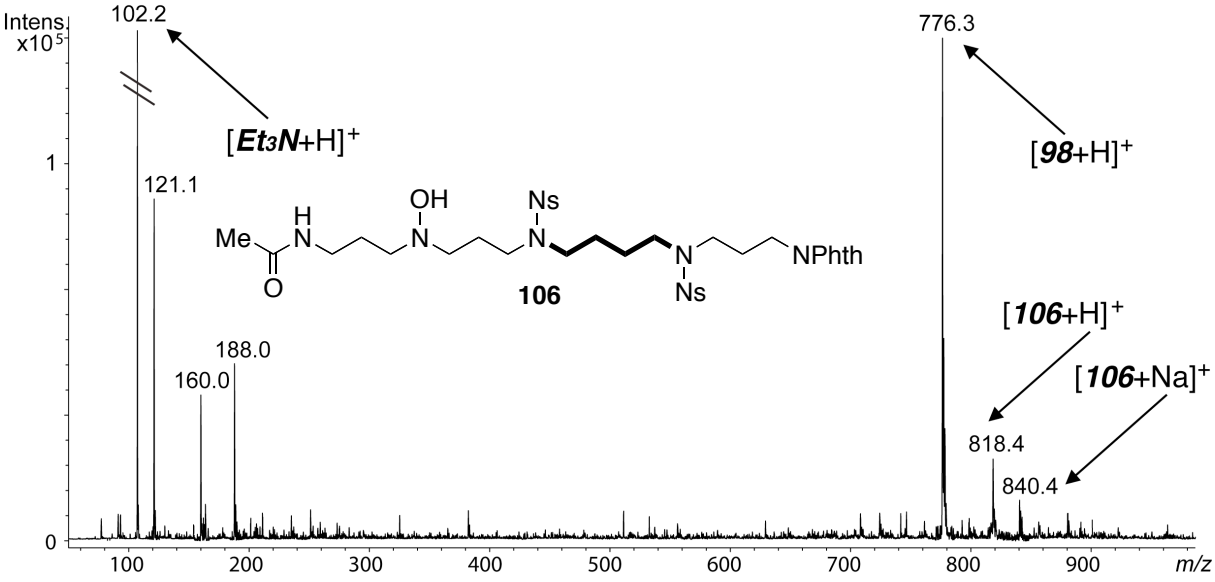


Figure 4. ESI-MS of crude **106**.

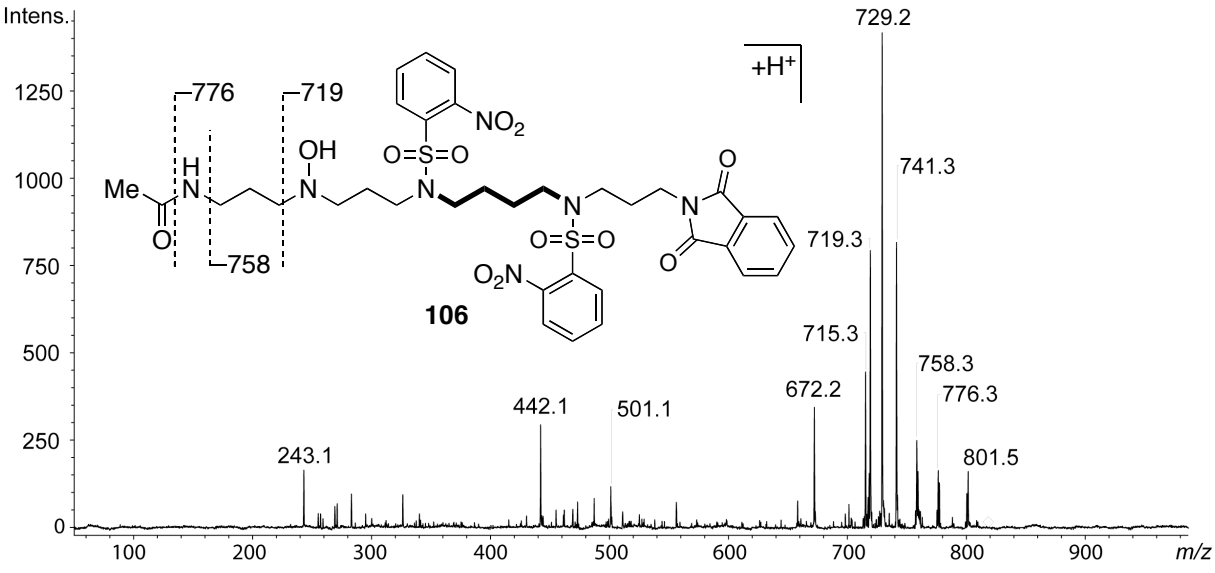


Figure 5. Confirmation of the structure of **106** by MS/MS of *m*/*z* 818.

The success of the acylation with acetyl chloride led us to the next attempt using benzoyl chloride as acylating reagent to be even closer to the structure of the chromophore of the natural product. Resin **105** was obtained after 7 h reaction time. After cleavage, crude **107** was measured by ESI-MS and it was demonstrated that the acylation reaction worked and was complete (Figure 6).

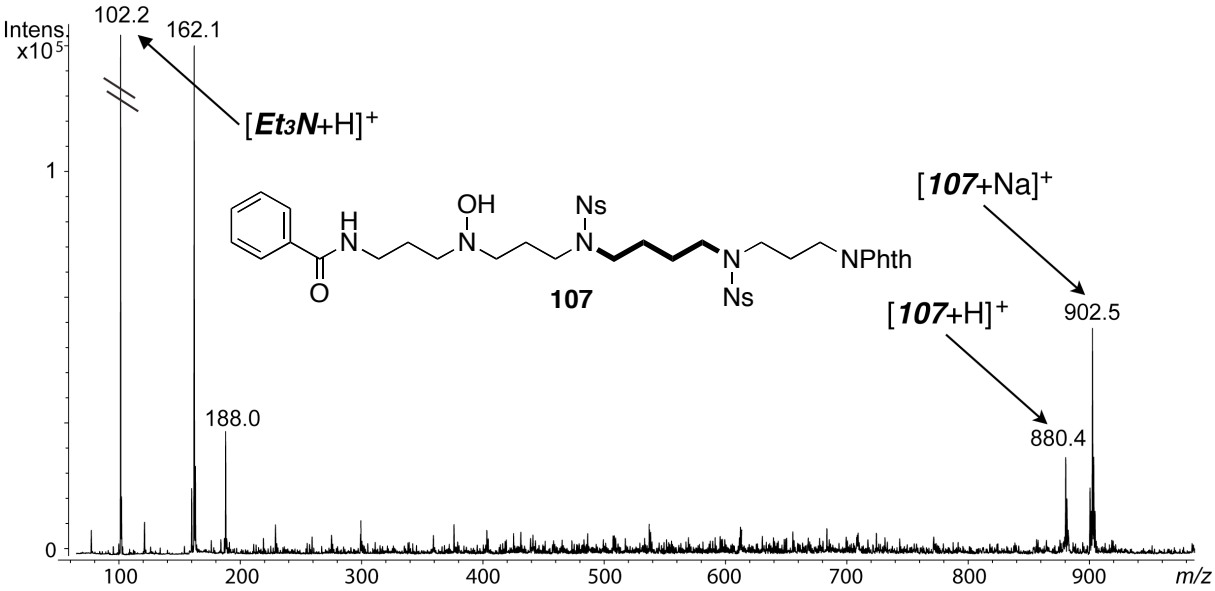


Figure 6. ESI-MS of crude **107**.

Tandem MS confirmed the structure of acylpolyamine **107** showing the same fragmentation patern as the acetyl polyamine **106** (Figure 5).

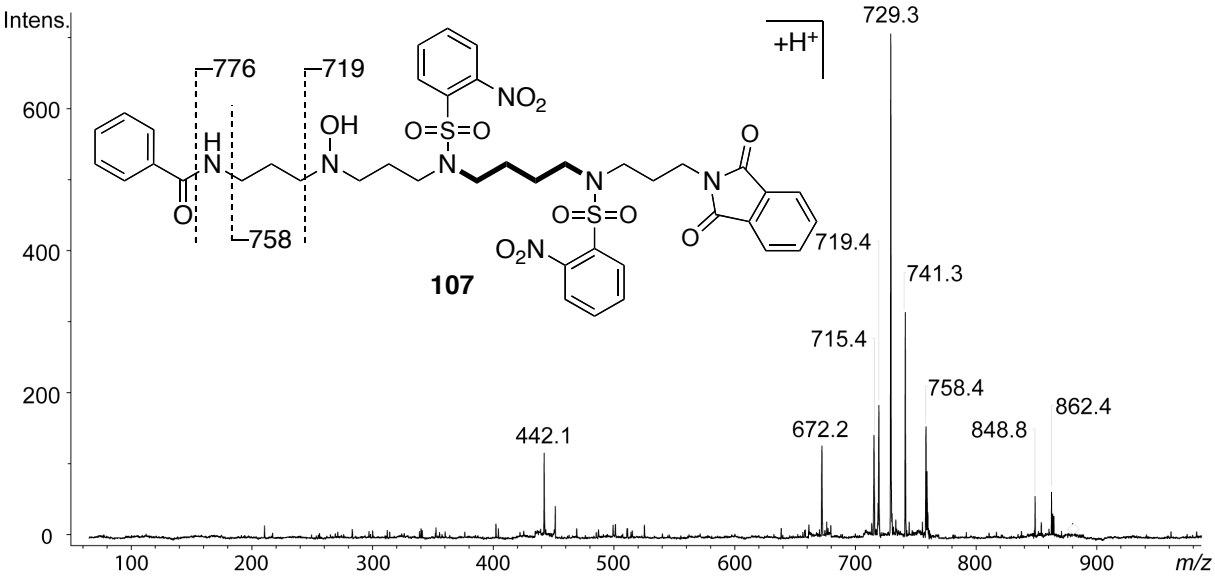
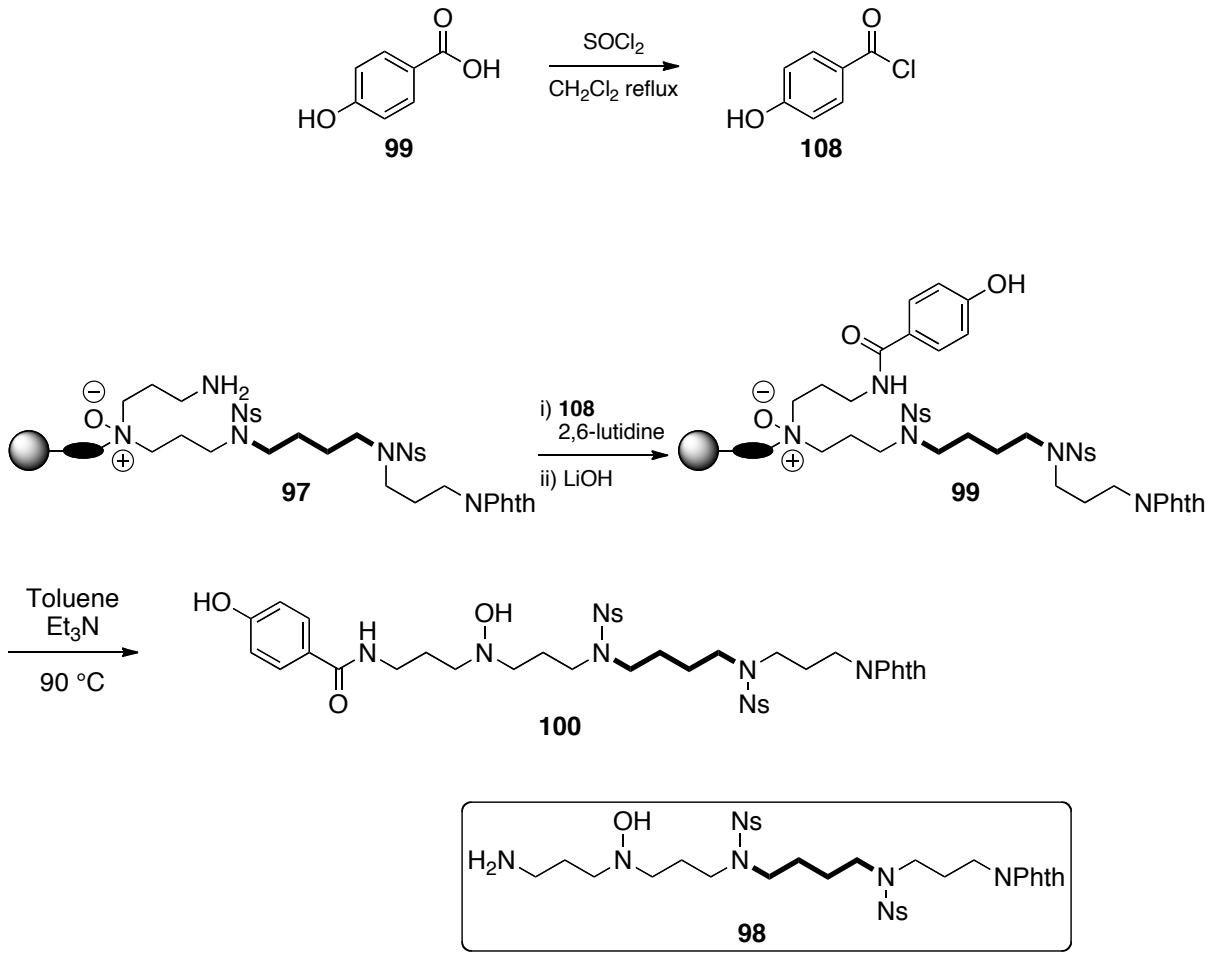


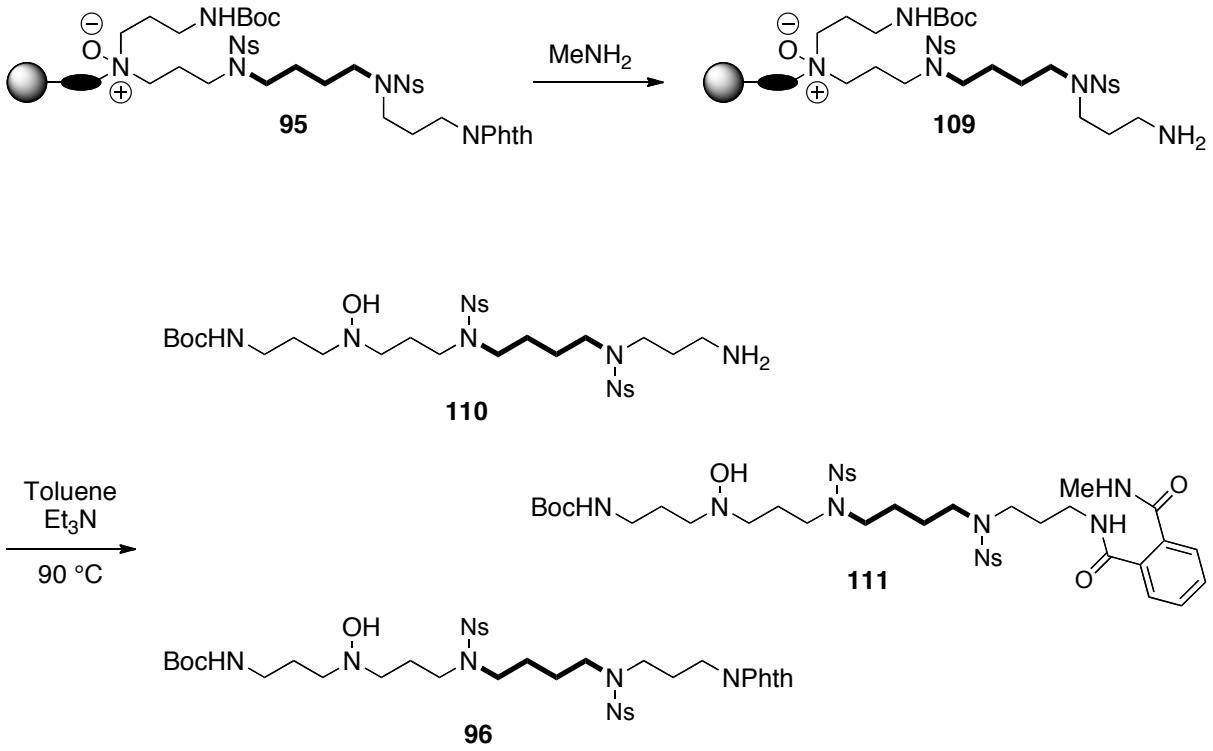
Figure 7. Confirmation of the structure of **107** by MS/MS of *m*/*z* 880.

These results led us to our last attempt. 4-Hydroxybenzoyl chloride (**108**) was prepared according to the procedure described by *Chao* et al.,[12] who prepared 3-formyl-4-hydroxybenzoyl chloride to be coupled to an amine functionalized resin. The carboxylic acid derivative **99** was converted into the corresponding acyl chloride in CH_2Cl_2 at reflux. Crude **108** was added to resin **97** in presence of 2,6-lutidine, a sterically hindered mild base, in CH_2Cl_2. The resulting resin **99** was submitted to cleavage conditions followed by ESI-MS measurements which indicated one more time that the acylation did not proceed at all since only the primary amine **98** was detected. These results show that further investigations need to be carried out in order to reveal the best conditions for the acylation of the primary amine position neighboring the resin.



Scheme 5. Acylation with 4-hydroxybenzoyl choride.

During our attempts to the preparation of the *Agelenopsis aperta* toxins we encountered some problems during the removal of the phthaloyl protective group (Scheme 6).



Scheme 6. Removal of the phthaloyl protective group.

As already mentioned, usual conditions with hydrazine at 80 °C could not be used since the temperature is very close to the one necessary to initiate the *Cope* elimination. The alternative method with methylamine at 23 °C showed to be efficient but not complete even after 50 h reaction time. Indeed after cleavage, non-reacted material (**96**), « half-deprotected » product (**111**) — addition of methylamine to the phthalimide moiety — and desired compound (**110**) were detected (Figure 8). In order to solve this problem, the reaction was run at 23 °C for 50 h followed by 2.5 h at 50 °C which provided a clean crude mixture containing only desired unprotected product.

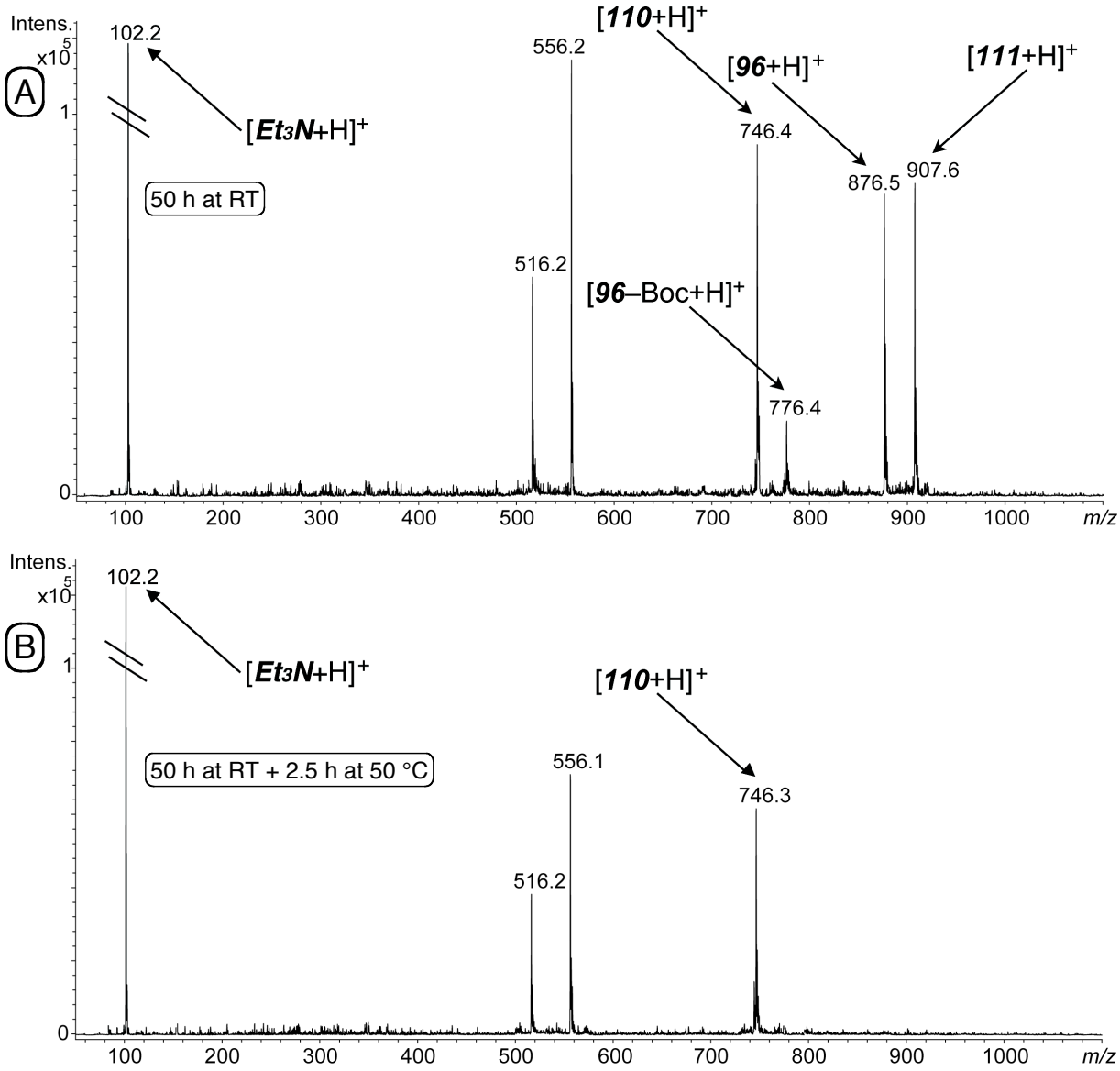


Figure 8. Optimisation of phthalimide group removal.

The two compounds at *m*/*z* 516 and 556, namely **112** and **113** (Figure 9), were identified by tandem MS as side products coming from wrong regioselectivity of the *Cope* elimination occurring on the polyamine backbone instead of on the linker moiety (**112**) or elimination by the sulfonyl moiety (**113**) (Scheme 7). The two side products presented the same MS/MS pattern, (1) loss of ammonia, corresponding to the signals at *m*/*z* 539 and 499, respectively for **112** and **113**, (2) the moiety containing the N-Ns group and four methylene units matching with the peak at *m*/*z* 257 and (3) the Ns group highlighted by the signal at *m*/*z* 186.

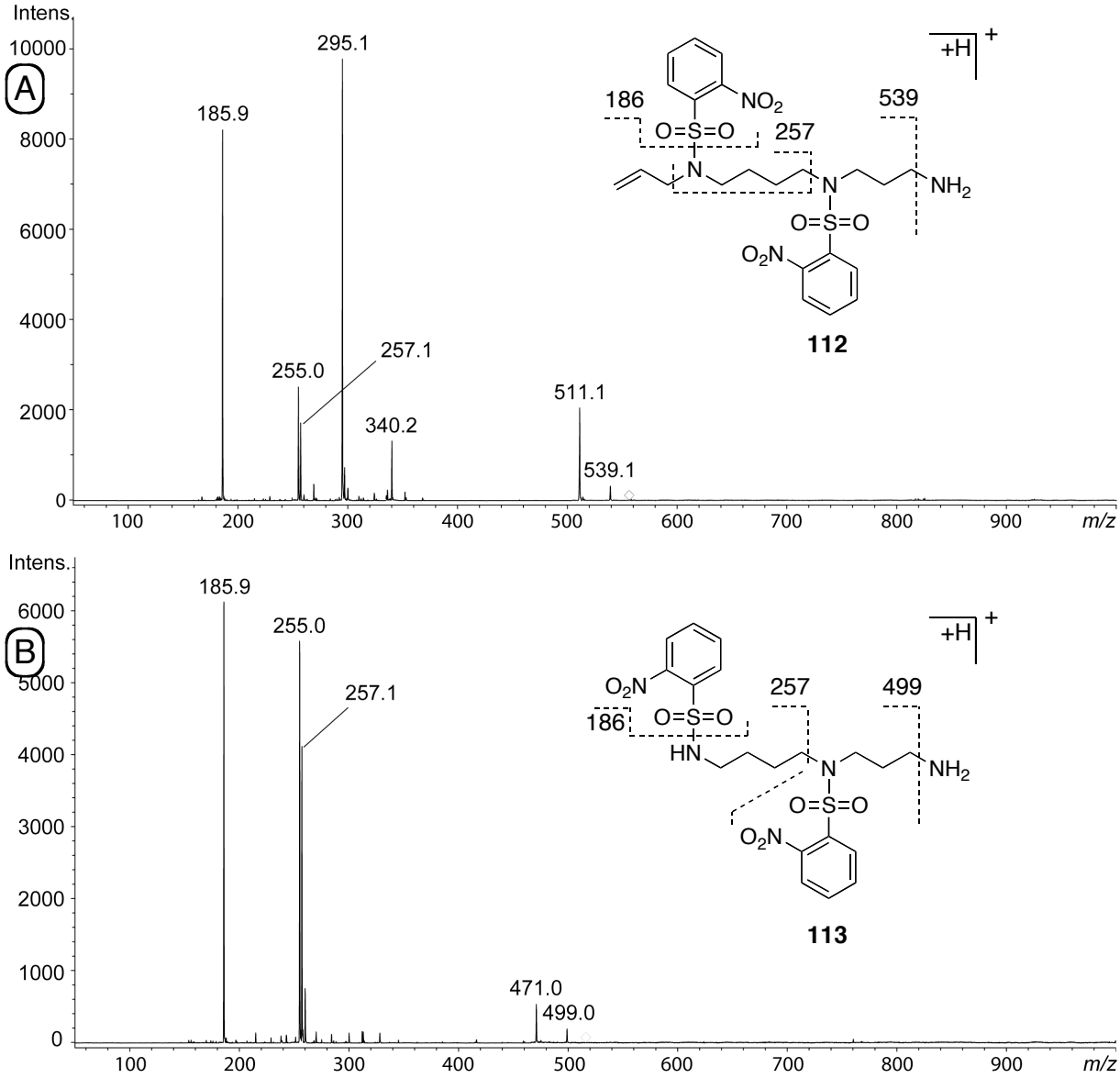
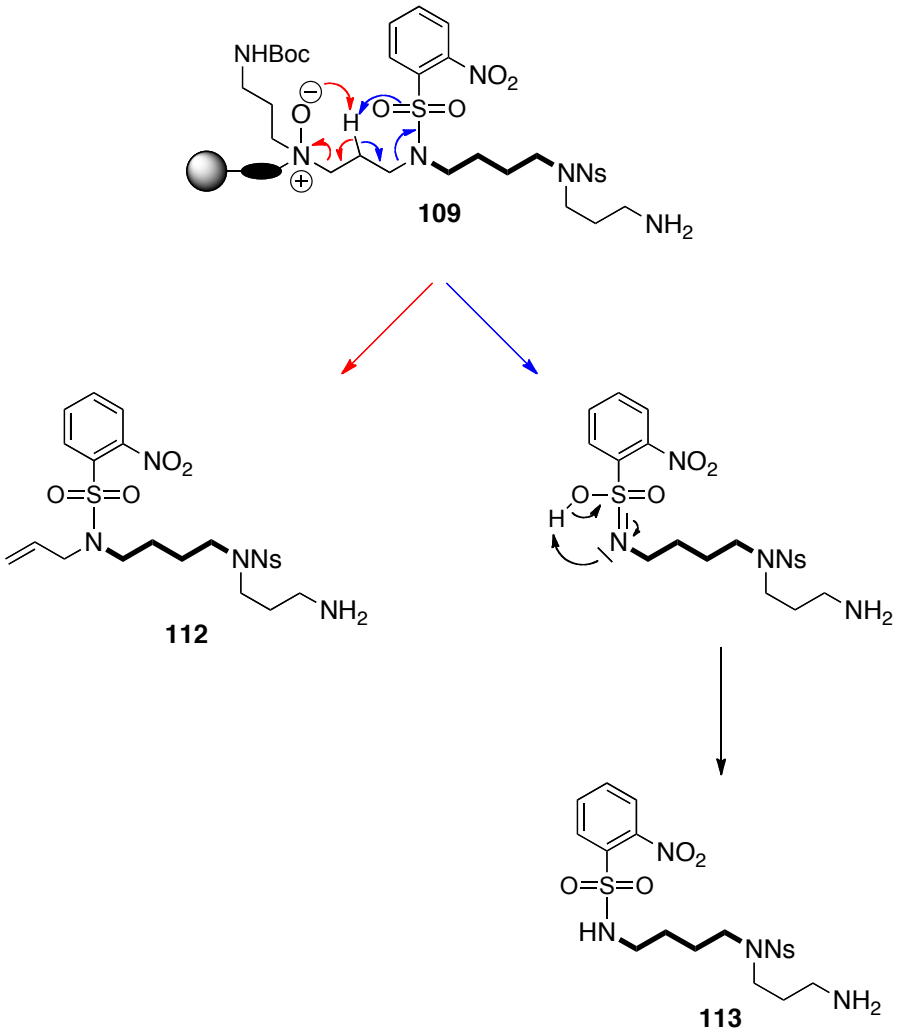


Figure 9. A) MS/MS of *m*/*z* 556. B) MS/MS of *m*/*z* 516.

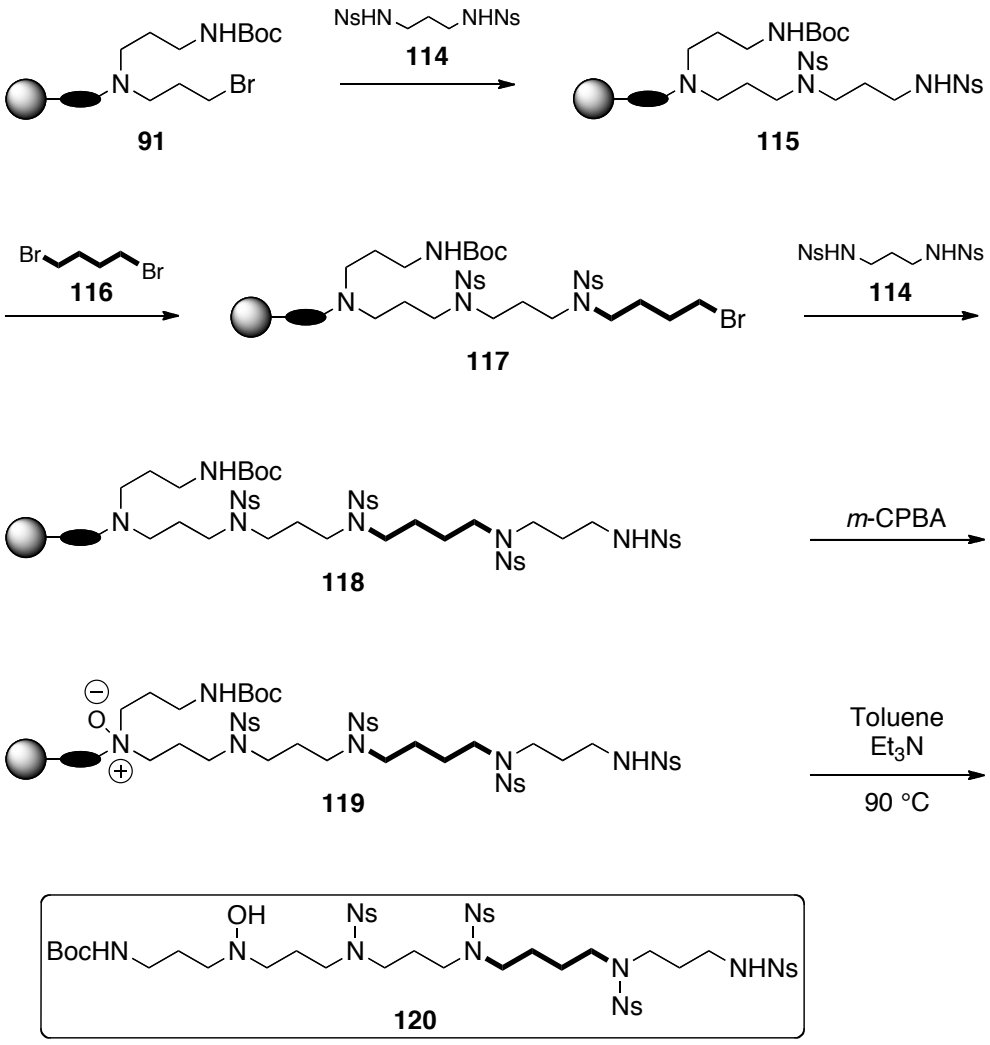
The observation of such undesired compounds during the cleavage step was the starting point of our investigations in the design of a new linker, able to avoid wrong regioselectivity of the *Cope* elimination but also able to give access to the synthesis of *N*-methylpolyamine derivatives *via Hofmann* elimination (see chapter 2).



Scheme 7. Formation mechanisms of **112** and **113**.

4. Synthesis of Hexaamine Derivative

The next target molecule was the *N*-hydroxylated hexaamine fully protected Boc3(OH)3Ns3Ns4Ns3Ns (**114**) (Scheme 8). The flexibility of our method is shown once more and is illustrated by the introduction of three consecutive portions of three methylene units. Starting from bromo resin **91**, the complete hexaamine backbone (**118**) was built by three successive nucleophilic substitution reactions using *N*,*N*-bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane **(114)**, 1,4-dibromobutane **(116)** and again di-Ns **114**. During this reactions sequence, the possibility of making cross-linking was present but the resulting products were never detected by ESI-MS after each elongation step.



Scheme 8. Solid-support preparation of the hexaamine backbone.

Hexaamine **120** was delivered after oxidation of an aliquot of resin **119** with *m*-CBPA followed by the *Cope* elimination in presence of Et_3N to avoid decomposition of the hydroxylamine to the secondary amine. The crude mixture was analysed by ESI-MS and the right product was detected confirming that the whole sequence of elongations worked correctly. The crude mixture was also measured by HR-ESI-MS to confirme that the signal that was detected by ESI-MS corresponds to the right expected compound. Unfortunately, during the measurement, we faced two difficulties (1) in-source reduction was noticed giving rise to signals observed previously during APCI-MS investigations (see Chapter 5) corresponding to $[M+H-18]^+$ and $[M+H-16]^+$. This artefact could be overcome by increasing the flow rate of

the infusion. (2) One peak of the isotopic pattern of an impurity was overlapping the desired signal consistent with [***120***+H]$^+$, creating a distortion of the signal that the instrument made an average of both peaks so that we could not get the high accuracy mass of hexaamine **120**. We then decided to record the high accuracy mass of the in-source induced reduced hexaamine (nitrone) at *m*/*z* 1169.26788 ([**120**–H_2–H]$^-$) which was in agreement with the chemical formula $C_{45}H_{57}N_{10}O_{19}S_4$ (calcd. 1169.26843).

5. CONCLUSIONS

The sequence, introduction of the linker – elaboration of the polyamine backbone – oxidation – deprotection – *Cope* elimination has been successfully applied to the preparation of penta- and hexaaamine derivatives, compounds which are structurally very close to the natural products present in the venom of the spider *Agelenopsis aperta*. Further investigations for the optimisation of the acylation step of the pathway will be realised to prepare spider toxins. These components will be used as model compounds for MS studies but also as supporting structures for confirmation of constituents of spider venom in addition of cytotoxicity assays and binding DNA experiments.

6. EXPERIMENTAL SECTION

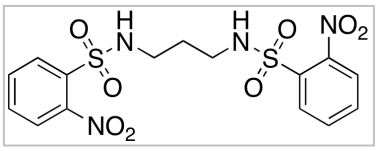
Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. Resin used: *Merrifield* polymer 200–400 mesh, 2% divinylbenzene, loading ~2.1 mmol Cl g^{-1} resin from *Fluka*. For the solid-phase reactions an *Advanced ChemTech PLS 6 Organic synthesiser* was used. IR spectra were recorded on a *Perkin–Elmer* 1600 Series FT-IR spectrophotometer and for the final products, an *OMNILAB* FT/IR 4100 spectrophotometer. Routine ^{1}H-NMR spectra in $CDCl_3$ or DMSO-d_6 were measured with a *Bruker* AC-300 (300 MHz); δ rel. to $CHCl_3$ (δ 7.26 ppm) or to DMSO (δ 2.50 ppm). Routine ^{13}C-NMR spectra in $CDCl_3$ were measured with a *Bruker* AC-300 (75.5 MHz); δ rel. to $CDCl_3$ (δ 77.0 ppm) or DMSO (δ 39.51 ppm). ESI-MS was performed on a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH*, Bremen, Germany), equipped with a combined *Hewlett-Packard* Atmospheric Pressure Ion (API) source (*Hewlett–Packard Co.*, Palo Alto, CA, USA). HR-MS: High-resolution electrospray mass spectra were recorded on a *Bruker MaXis* QTOF-MS instrument (*Bruker Daltonics GmbH*, Bremen, Germany). The samples were dissolved in MeOH and analyzed *via* continuous flow injection at 3 μL min^{-1}. The mass spectrometer was operated in positive ion mode with a capillary voltage of 4 kV, an endplate offset of –500 V, nebulizer pressure of 5.8 psi, and a drying gas flow rate of 4 L min^{-1} at 180 °C. The mass spectrometer was operated in negative ion mode with a capillary voltage of –3.5 kV, an endplate offset of –500 V, nebulizer pressure of 5.8 psi, and a drying gas flow rate of 4 L min^{-1} at 180 °C. The instrument was calibrated with a *Fluka* electrospray calibration solution (*Sigma-Aldrich*, *Buchs*, Switzerland) that has been 100 times diluted with MeCN. The resolution was optimised at 30'000 FWHM in the active focus mode. The accuracy was better than 2 ppm in a mass range between *m*/*z* 118 and 2721. All solvent used were purchased in best LC-MS qualities.

6.1. Preparation of the Building Blocks

***tert*-Butyl *N*-(3-Aminopropyl)carbamate (88).** To a solution of 1,3-diaminopropane (19.7 mL, 235.8 mmol) in dioxane (100 mL) under an Ar atmosphere, di-*tert*-butyldicarbonate (17.15 g, 78.6 mmol) dissolved in dioxane (50 mL) was added dropwised at 0 °C. The reaction was stirred overnight at 23 °C then reflux (120 °C) for 4 h. The mixture was filtered off and the fliltrat was concentrated *in vacuo* to give a yellow oil. Purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 (25%) 1:0.1:0.01) gave **88** as a slightly yellow oil (10.26 g, 58.9 mmol, 75% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ = 3.24–3.17 (m, 2 H, BocNHCH_2), 2.69 (t, $^3J_{H,H}$ = 6.6 Hz, 2 H, NH_2CH_2), 1.61 (quint, 2 H, $^3J_{H,H}$ = 6.6 Hz, $CH_2CH_2CH_2$), 1.44 (s, 9 H, Me_3C) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ = 156.3 (s, CO), 79.1 (s, Me_3C), 39.9, 33.6 (2 t, 2 CH_2), 28.6 (q, Me_3C), 28.5 (t, CH_2) ppm. ESI-MS m/z = 197.0 (20, $[M+Na]^+$), 175.1 (100, $[M+H]^+$), 119.1 (46, $[M-56+H]^+$).

***N*,*N*-Bis-(2-nitrophenylsulfonyl)-1,4-diaminobutane (92).** To a solution of 1,4-diaminobutane (3.91 g, 44.4 mmol) and Et_3N (12.48 mL, 88.8 mmol) in dry CH_2Cl_2 (70 mL) at 0 °C was added dropwise a solution of 2-nitrophenylsulfonyl chloride (19.67 g, 88.8 mmol) in dry CH_2Cl_2 (70 mL). The mixture was stirred at 23 °C for 3 h. The precipitate was filtered off and whashed with CH_2Cl_2 and H_2O. The slightly yellow solid was dried *in vacuo* (19.38 g, 42.3 mmol, 95% yield). ^{1}H-NMR (300 MHz, DMSO-d_6, 298 K) δ = 7.99–7.82 (m, 8 H, arom. H), 3.30 (*br.* s, 2 H, NsNH), 2.85 (*br.* t, $^3J_{H,H}$ = 5.9 Hz, 4 H, NsNHCH_2), 1.42 (*br.* quint, $^3J_{H,H}$ = 3.2 Hz, 4 H, $NsNHCH_2$-CH_2) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6, 298 K) δ = 147.7 (s, 2 arom. CNO_2), 133.8 (d, 2 arom. CH *p* to NO_2), 132.7 (s, 2 arom. CSO_2), 132.5 (d, 2 arom. CH *p* to SO_2), 129.3 (d, 2 arom. CH *o* to NO_2), 124.3 (d, 2 arom. CH *o* to SO_2), 42.2 (t, 2 $NsNHCH_2$), 26.2 (t, 2 $NsNHCH_2CH_2$) ppm. ESI-MS m/z = 457.0 (100, $[M-H]^-$).

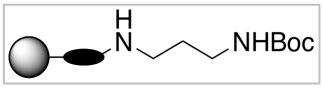
***N,N*-Bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane (114).** To a solution of



1,3-diaminopropane (3.77 mL, 45.1 mmol) and Et_3N (12.93 mL, 90.2 mmol) in dry CH_2Cl_2 (70 mL) at 0 °C was added dropwise a solution of 2-nitrophenylsulfonyl chloride (19.99 g, 90.2 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred at 23 °C for 3 h. The precipitate was filtered off and whashed with CH_2Cl_2 and H_2O. The colourless solid was dried *in vacuo* (18.62 g, 41.9 mmol, 93% yield). ^{1}H-NMR (300 MHz, DMSO-d_6, 298 K) δ = 8.18–8.13 (m, 4 H, arom. H), 8.09–8.03 (m, 4 H, arom. H), 3.11 (t, $^3J_{H,H}$ = 7.1 Hz, 4 H, NsNHCH_2), 1.80 (quint, $^3J_{H,H}$ = 7.1 Hz, 2 H, NsNHCH$_2$CH_2) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6, 298 K) δ = 147.6 (s, 2 arom. CNO_2), 133.8 (d, 2 arom. CH *p* to NO_2), 132.7 (s, 2 arom. CSO_2), 132.4 (d, 2 arom. CH *p* to SO_2), 129.2 (d, 2 arom. CH *o* to NO_2), 124.2 (d, 2 arom. CH *o* to SO_2), 42.1 (t, 2 NsNHCH$_2$), 26.1 (t, 2 NsNHCH$_2$CH$_2$) ppm. ESI-MS *m*/*z* = 443.3 (100, [*M*–H]$^-$).

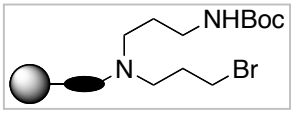
6.2. Synthesis of Solid-Supported Penta- and Hexaamine Derivatives

Introduction of the Linker and Alkylation with *tert*-Butyl *N*-(3-Aminopro-

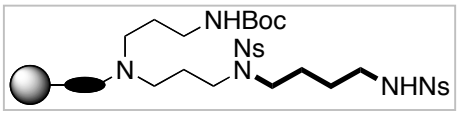


pyl)carbamate (88) to Form Resin 89. MeONa (2.43 g, 45.0 mmol) was added to a solution of 2-(4-hydroxyphenyl)ethanol (6.21 g, 45.0 mmol) in NMP (80 mL) at 0 °C. The solution was warmed up to 23 °C, mechanically stirred for 2 h, and the *Merrifield* resin (9.39 g, 15.0 mmol, loading capacity determined by *Volhard* titration:[13] 1.6 mmol Cl g^{-1}) was added. The resulting suspension was mechanically stirred for 18 h at 50 °C. The resin was filtered off, washed successively with DMF, CH_2Cl_2, and MeOH, and dried *in vacuo*. *Volhard* titration confirmed the completion of the reaction. IR ν = 3350 (*br.*, OH) cm^{-1}. The resulting resin (10.89 g, 15.0 mmol) was swelled in dry CH_2Cl_2 (80 mL) for 15 min. PPh_3 (6.56 g, 25.0 mmol) was added. The mixture was cooled down to 0 °C followed by the slow addition of CBr_4 (24.87 g, 75.0 mmol). The suspension was warmed up to 23 °C and mechanically stirred for 12 h under Ar atmosphere. The resin was filtered off, washed successively with DMF, CH_2Cl_2, and MeOH and dried *in vacuo*. Loading: 1.6 mmol g^{-1} (100%). The resulting resin (15.0

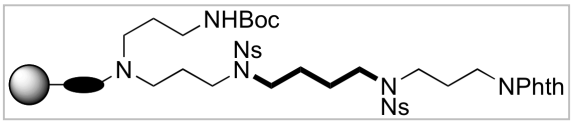
mmol, 1.6 g mol^{-1} as determined by *Volhardt* titration) was swelled in NMP (30 mL) for 15 min. DIEA (12.84 mL, 75.0 mmol), *N*-(3-aminopropyl)carbamate (**88**, 13.07 g, 75.0 mmol) were added, and the suspension was agitated for 24 h at 50 °C. Resin **89** was filtered off, washed successively with NMP, CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 1707 (CO) cm^{-1}.

Alkylation of Resin 89 with 1,3-Dibromopropane (90) to Form Resin 91 (General Procedure (1)). 

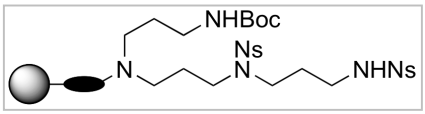
Resin **89** (9.5 mmol) was swelled in NMP (20 mL) at 50 °C for 15 min. 1,3-dibromopropane (**90**, 4.84 mL, 47.5 mmol) and DIEA (8.13 mL, 47.5 mmol) were added and the suspension was agitated for 24 h at 50 °C. Resin **91** was filtered off, washed successively with NMP, CH_2Cl_2 and MeOH and dried *in vacuo*. IR ν = 1671 (CO) cm^{-1}.

Alkylation of Resin 91 with *N*,*N*-Bis-(2-nitrophenylsulfonyl)-1,4-diaminobutane (92) to Form Resin 93 (General Procedure (2)). 

Resin 91 (3.0 mmol) was swelled in DMF (20 mL) at 50 °C for 15 min. Cs_2CO_3 (7.31 g, 15.1 mmol) and *N*,*N*-bis-(2-nitrophenylsulfonyl)-1,4-diaminobutane (92, 4.91 g, 15.1 mmol) were added, and the suspension was agitated for 24 h at 50 °C. Resin 93 was filtered off, washed successively with DMF, NMP/H_2O (1:1), NMP, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1681 (CO), 1509 (NO_2), 1365 (SO_2), 1162 (SO_2) cm^{-1}.

Alkylation of Resin 93 with *N*-(3-Bromopropyl)phthalimide (23) to Form Resin 94. 

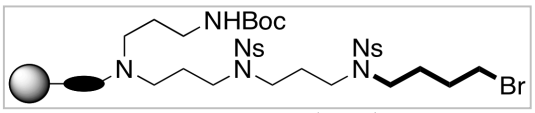
According to the general procedure (2), resin **93** (3.0 mmol) was elongated with *N*-(3-bromopropyl)-phthalimide (**23**) to give resin **94**. IR ν = 1770 (CO of Phth), 1712 (CO of Boc/Phth), 1509 (NO_2), 1452 (SO_2), 1369(NO_2), 1160 (SO_2) cm^{-1}.

Alkylation of Resin 91 with *N*,*N*-Bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane (114) to Form Resin 115. 

According to the general procedure (2), resin **91** (2.0 mmol) was

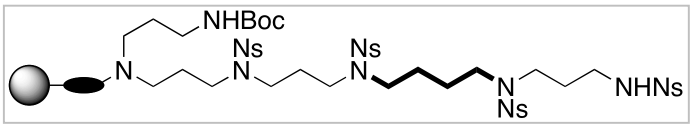
elongated with *N*,*N*-bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane (**114**) to give resin **115**. IR ν = 1680 (CO), 1509 (NO_2), 1369 (SO_2), 1162 (SO_2) cm^{-1}.

Alkylation of Resin 115 with 1,4-Dibromobutane (116) to Form Resin 117.



According to the general procedure (1), resin **115** (0.2 mmol) was elongated with 1,4-dibromobutane (**116**) to give resin **117**. IR ν = 1681 (CO), 1509 (NO_2), 1365 (SO_2), 1160 (SO_2) cm^{-1}.

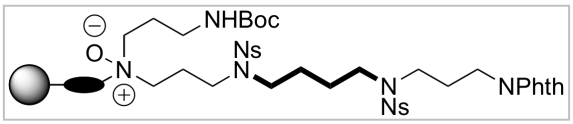
Alkylation of Resin 117 with *N*,*N*-Bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane (114) to Form Resin 118.



According to the general procedure (2), resin **117** (0.2 mmol) was elongated with *N*,*N*-bis-(2-nitrophenylsulfonyl)-1,3-diaminopropane (**114**) to give resin **118**. IR ν = 1681 (CO), 1507 (NO_2), 1363 (SO_2), 1162 (SO_2) cm^{-1}.

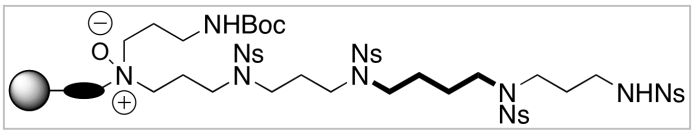
6.3. Oxidation of the Solid-Supported Polyamine Derivatives

Oxidation of Resin 94 with *m*-CPBA to Form Resin 95 (General Procedure).



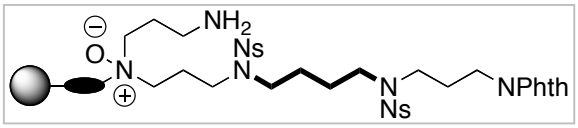
Resin **94** (3.0 mmol) was swelled in CH_2Cl_2 (5 mL) for 15 min. *m*-CPBA (0.60 g, 15.1 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added, and the suspension was agitated for 3 h at 23 °C. Resin **95** was filtered off, washed successively with DMF, MeOH and CH_2Cl_2 and dried *in vacuo*. IR ν = 1770 (CO of Phth), 1711 (CO of Phth/Boc), 1509 (NO_2), 1366 (SO_2), 1160 (SO_2) cm^{-1}.

Oxidation of Resin 118 with *m*-CPBA to Form Resin 119. According to the general procedure, resin **118** (0.2 mmol) was oxidised with *m*-CPBA to give resin **119**. IR ν = 1770 (CO of Phth), 1711 (CO of Phth/Boc), 1509 (NO_2), 1366 (SO_2), 1160 (SO_2) cm^{-1}.



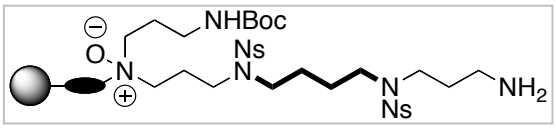
6.4. Removal of the Protective Groups

Removal of the Boc Protective Group of Resin 95 to Form Resin 97. Resin **95**



(0.5 mmol) was swelled in CH_2Cl_2 (3 mL) for 15 min. TFA (0.76 mL, 10.0 mmol) was added and the suspension was agitated for 12 h at 23 °C. Resin **97** was filtered off and washed successively with CH_2Cl_2, CH_2Cl_2/DIEA (1:1), CH_2Cl_2, and MeOH and dried *in vacuo*. The *Kaiser* test[7] was performed to prove the presence of primary amine group. IR ν = 1770 (CO), 1709 (CO), 1509 (NO_2), 1369 (SO_2), 1163 (NO_2) cm^{-1}.

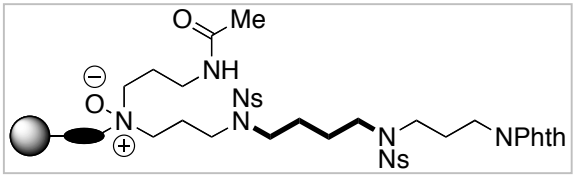
Removal of the Phth Protective Group of Resin 95 to Form Resin 109. Resin



95 (0.1 mmol) was swelled in NMP (2 mL). Methylamine (41% in H_2O, 75 μL, 1.00 mmol) was added. The suspension was agitated at 23 °C for 50 h and for additional 2.5 h at 50 °C. Resin **109** was filtered off and washed successively with NMP (2×), NMP/H_2O (1:1), NMP, DMF, CH_2Cl_2 and MeOH and dried *in vacuo*. The *Kaiser* test[7] was performed to prove the presence of primary amine group. IR ν = 1680 (CO), 1509 (NO_2), 1365 (SO_2), 1162 (SO_2) cm^{-1}.

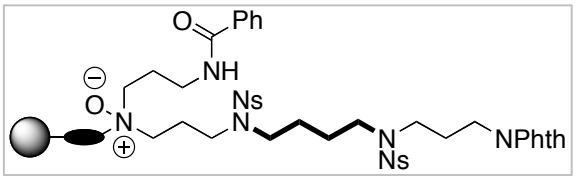
6.5. Acylation of the Solid-Supported Polyamine Derivatives

Acylation of Resin 97 with Acetyl Chloride to Form Resin 104. Resin **97**



(0.2 mmol) was swelled in CH_2Cl_2. Acetyl chloride (0.14 mL, 2.00 mmol) and Et_3N (0.28 mL, 2.0 mmol) were added. The suspension was agitated at 23 °C for 3 h. Resin **104** was washed successively with CH_2Cl_2, CH_2Cl_2/DIEA (1:1), CH_2Cl_2, and MeOH and dried *in vacuo*. The *Kaiser* test[7] was performed to prove the absence of primary amine group. IR ν = 1638 (COMe), 1770 (CO of Phth), 1709 (CO of Phth), 1509 (NO_2), 1369 (SO_2), 1162 (SO_2) cm^{-1}.

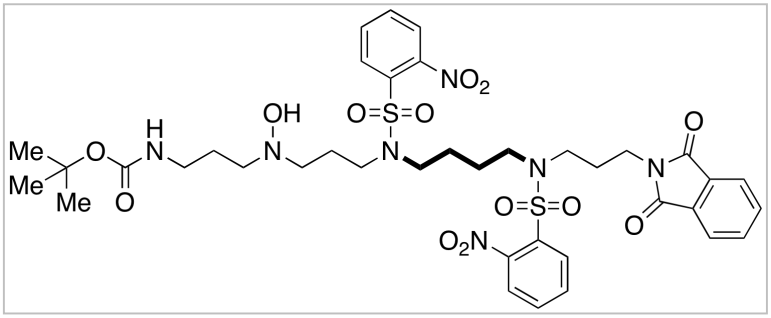
Acylation of Resin 97 with Benzoyl Chloride Form Resin 105. Resin **97** (0.2 mmol) was swelled in CH_2Cl_2. Benzoyl chloride (0.23 mL, 2.00 mmol) and Et_3N (0.28 mL, 2.0 mmol) were added. The suspension was agitated at 23 °C for 7 h. Resin **105** was washed successively with CH_2Cl_2, CH_2Cl_2/DIEA (1:1), CH_2Cl_2, and MeOH and dried *in vacuo*. The *Kaiser* test[7] was performed to prove the absence of primary amine group. IR $\nu = 1663$ (COPh), 1770 (CO of Phth), 1711 (CO of Phth), 1509 (NO_2), 1366 (SO_2), 1160 (SO_2) cm^{-1}.



6.6. Liberation of the Pentaamine Derivatives from the Resins

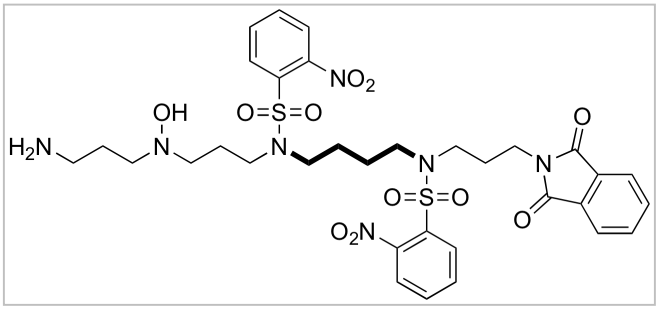
General Procedure for the *Cope* Elimination: The resin (obtained from 0.2 mmol of resin **2**) was swelled in toluene (5 mL). Et_3N (3 drops) was added. The suspension was agitated for 2 h at 90 °C. The resin was filtered off and washed with toluene, CH_2Cl_2 and MeOH. The combined filtrates were evaporated to give a yellow oil.

***tert*-Butyl *N*-[4-Hydroxy-8,13-di-(2-nitrophenylsulfonyl)-17-phthalimido-4,8, 13-triazaheptadecyl]carbamate (96) from Resin 95.**



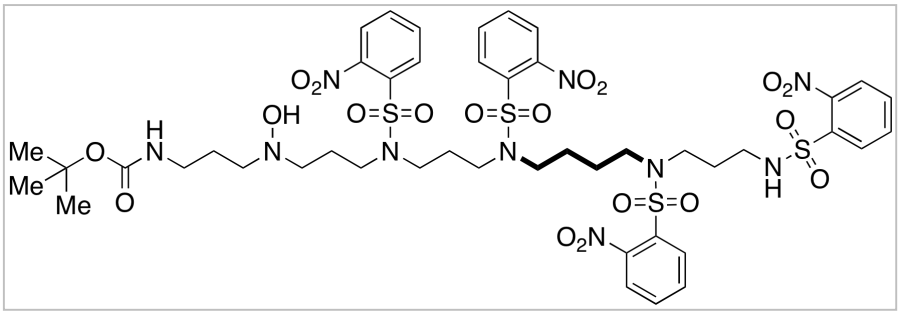
HR-MS: calcd. For $C_{38}H_{50}N_7O_{13}S_2$ 876.29025; found 876.29087.

***N*-[16-Amino-13-hydroxy-4,9-di-(2-nitrophenylsulfonyl)-4,9,13-triazahexadecyl]phthalimide (98) from Resin 97.**



HR-MS: calcd. for $C_{33}H_{42}N_7O_{11}S_2$ 776.23782; found 776.23742.

***tert*-Butyl *N*-[4-Hydroxy-8,13-di-(2-nitrophenylsulfonyl)-17-phthalimido-4,8, 13-triazaheptadecyl]carbamate (120) from Resin 119.**



HR-MS of the corresponding nitrone: calcd. for $C_{45}H_{57}N_{10}O_{19}S_4$ 1169.26843; found 1169.26788.

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— Chapter 5 —

Decomposition of *N*-Hydroxylated Compounds During Atmospheric Pressure Chemical Ionization*

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are two of the most important ionization techniques used in mass spectrometry. These methods are of particular importance for analytical setups in which mass spectrometry is on-line coupled to liquid chromatography (LC-MS). Since ESI and APCI are mild ionization methods, they typically produce quasi-molecular ions of the analytes without fragmentation. Hence, they generate ions that usually provide direct and unequivocal information about the masses of the sample molecules. Such information, however, is convoluted by analytes that undergo fragmentations or decompositions prior to the MS analysis, e.g., before or during the ionization process. Formation of such artifacts, especially when their source is not known, might lead to misinterpretation of MS data. An understanding of potential decomposition reactions that might occur with analytes prior to their entry into the MS is particularly important for more complex investigations, e.g., for the investigation of natural products.

Artifact formation has already been observed with APCI, in which the analyte solutions are typically heated to 300–400 °C prior to ionization and therefore prone to thermally induced reactions. For example aromatic nitro compounds,[1,2] *N*-oxides,[3-7] and imines[8] all showed partial reduction to the corresponding amines upon APCI. An analogeous reaction was recently also observed with *N*-hydroxylated compounds in the course of our investigations

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of spider venoms.[9-12] Here we report that *N*-hydroxylated polyamine derivatives also undergo reduction to the respective amines too. This reaction has not previously been reported in literature. These results are significant because (1) *N*-hydroxylamines are well-known constituents of natural samples and also potential metabolites of drugs and (2) LC-APCI-MS is widely used for the study of natural products and drug metabolism. Thus, the lack of awareness of this APCI-reduction could broadly lead to wrong conclusions. To obtain means to recognize and control artifact formation from *N*-hydroxylamine by APCI, we investigated this in-source reaction with a synthetic *N*-hydroxylated polyamine derivative in depth.

1. Investigations with Synthetic *N*-Hydroxylated Compound

Tetraamine derivative **41** (Figure 1), which was prepared during our synthetic pursuit of *N*-hydroxylated polyamine spider toxins,[13] was chosen as the model compound for our study of the APCI behavior of *N*-hydroxylated secondary amines. The compound readily undergoes the investigated decomposition reactions and is also accessible in pure form. Compound **41** also contains an additional nitroaryl group, allowing the concurrent study of the decomposition of *N*-hydroxy and aromatic nitro[1] functionalities.

The investigation of the MS behavior of compound **41** started with two LC-MS runs performed with either an ESI or an APCI source under conditions previously applied for the analyses of polyamine spider toxins.[9,10,14,15] The LC-ESI-MS spectrum of the chromatographic peak of **41** (Figure 1, A) showed the expected signal for the protonated molecule $[M+H]^+$ at *m*/*z* 664 (base peak) and a weaker signal assigned to ions of the type $[M+Na]^+$ (*m*/*z* 686, 7%). Two additional signals at *m*/*z* 648 (4%) and *m*/*z* 646 (1%) were also observed corresponding to ions of the type $[M+H-16]^+$ and $[M+H-18]^+$, respectively. Often, ion signals with such low relative intensities are ignored. These signals, however, became relevant when APCI was used instead of ESI as the ionization method. Namely, the spectrum obtained by LC-APCI-MS (Figure 1, B) showed $[M+H-16]^+$ at *m*/*z* 648 (base peak) and $[M+H-18]^+$ at *m*/*z* 646 (18%)

with significantly higher relative intensities. A third signal that cannot be ignored was found at *m*/*z* 662 (27%), corresponding to $[M+H-2]^+$ ions. Since it was shown by LC-UV(DAD) and by NMR that the sample compound was pure, these three additional ions had to be generated by in-source decomposition of **41,** and were not due to impurities.

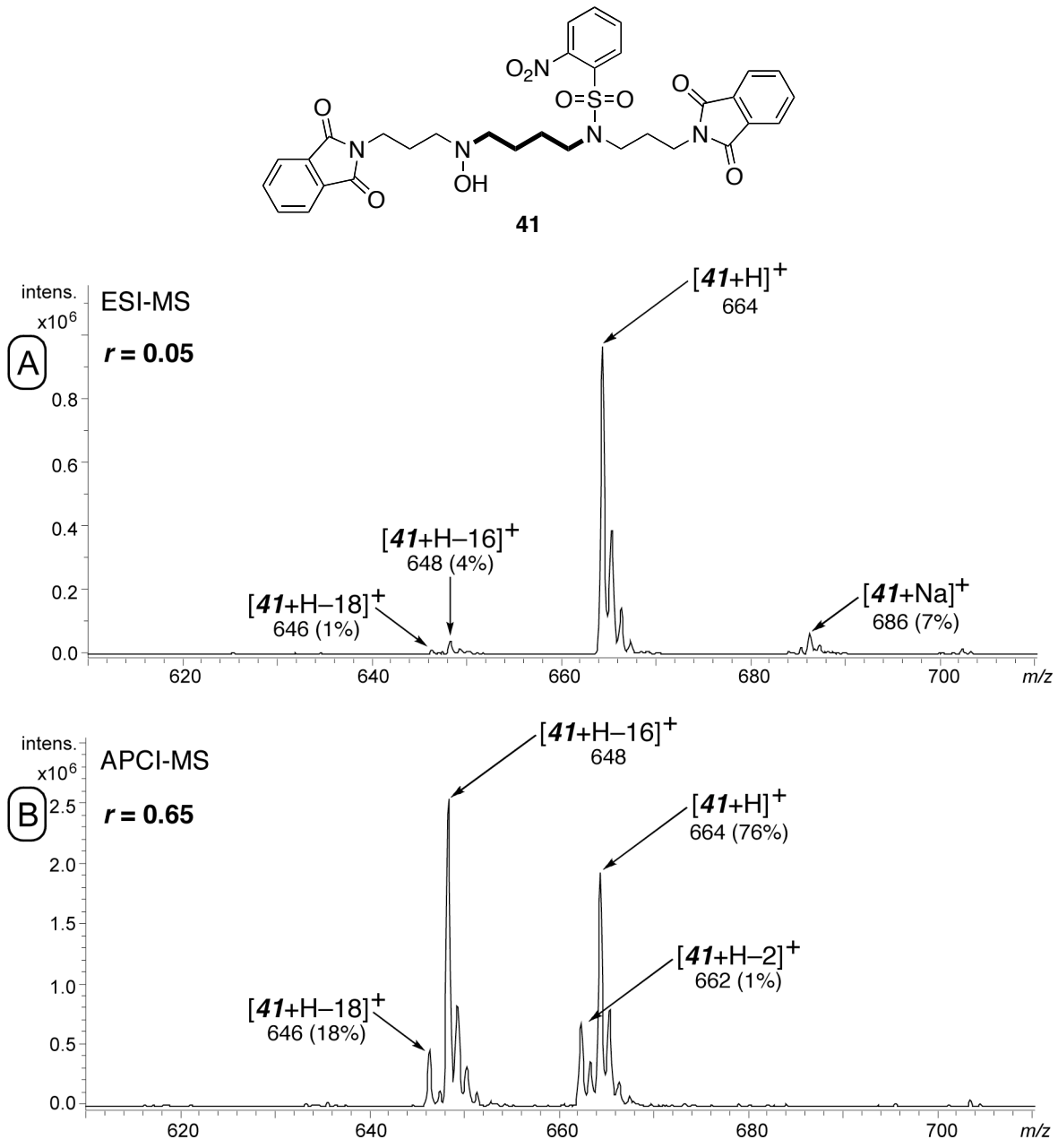

Figure 1. Structure and (A) LC-ESI-MS and (B) LC-APCI-MS of *N*-hydroxylated tetraamine derivative **41** in MeCN/H_2O + 0.1% TFA.

A term *r* with the following equation is introduced to estimate the extent of the overall decomposition of **41** occurring in the different experiments:

$$r = \frac{\sum I_{DP_i}}{I_{QMI} + \sum I_{DP_i}} = \frac{I_{[M+H-2]^+} + I_{[M+H-16]^+} + I_{[M+H-18]^+}}{I_{[M+H]^+} + I_{[M+H-2]^+} + I_{[M+H-16]^+} + I_{[M+H-18]^+}}$$

In this equation, I_{DP_i} corresponds to signal intensities of the decomposition products and I_{QMI} to signal intensity of the quasi-molecular ions. Although *r* is not the actual molecular ratio of the three decomposition products to the initial amount of **41**, it can still be regarded as a qualitative measure to describe the extent of decomposition of **41**, allowing, therefore, the characterization of different experiments.

The structures of the three artifactual ions at *m*/*z* 648, 662, and 646 were deduced from data acquired by LC-APCI-MS/MS and from their accurate masses measured by high-resolution ESI-MS of sample compound **41**. The exact masses of $[M+H]^+$, $[M+H-16]^+$, and $[M+H-18]^+$ revealed that the artifacts were generated by formal loss of O and H_2O from **41**, respectively (Table 1). No high-resolution ESI-MS data was available for the signal at *m*/*z* 662 ($[M+H-2]^+$) because its intensity was too low. However, the loss of H_2 from the parent compound **41** appears to be the most reasonable process that could generate an artifact responsible for the respective signal.

ion signal	elem. comp.	*m*/*z* theor.	*m*/*z* meas.	Δ ppm
$[\mathbf{41}+H]^+$	$C_{32}H_{34}O_9N_5S$	664.20717	664.20727	0.1
$[\mathbf{41}+H-O]^+$	$C_{32}H_{34}O_8N_5S$	648.21226	648.21248	0.4
$[\mathbf{41}+H-H_2O]^+$	$C_{32}H_{32}O_8N_5S$	646.19661	646.19692	0.5

Table 1. Exact masses obtained from high resolution ESI-FT-MS investigation of **41**.

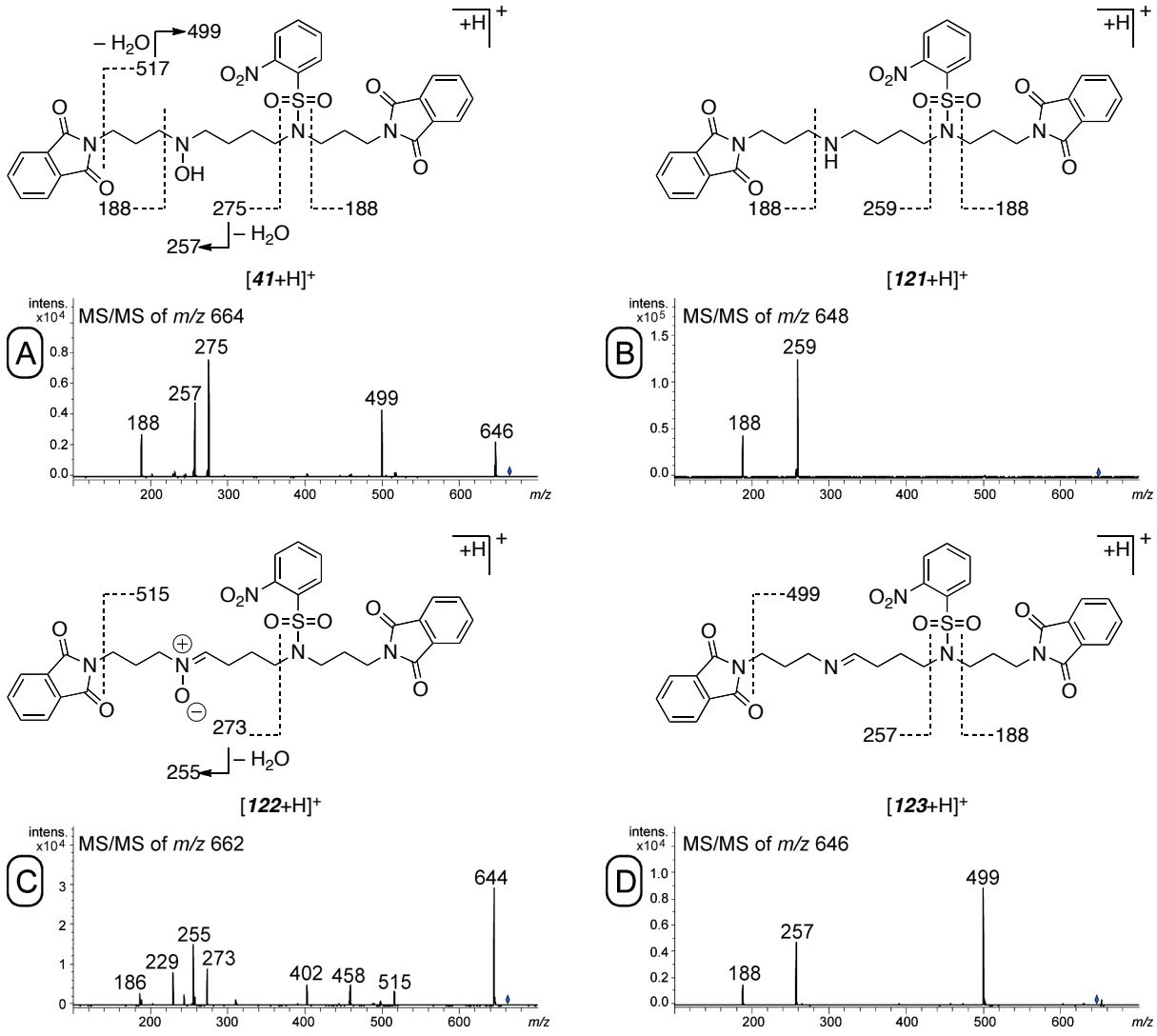

Figure 2. LC-APCI-MS/MS of (A) [***41***+H]$^+$, (B) [***41***+H–16]$^+$ = [***121***+H]$^+$, (C) [***41***+H–2]$^+$ = [***122***+H]$^+$, and (D) [***41***+H–18]$^+$ = [***123***+H]$^+$ ions with proposed structures and assignments of relevant fragment ions. For the nitrones ***122*** and the imines ***123***, only one of the two isomeric forms are shown.

The structures of the artifacts formed in the ion source were deduced from their MS/MS data as amine **121**, nitrone **122**, and imine **123** (in protonated forms, Figure 2). The loss of oxygen from **41** could have principally occured either at the N-OH position or at the NO_2/SO_2 groups of the Ns portion. The data revealed, however, that reduction only took place at the hydroxylamine functionality, thus forming product **121**. While fragment ions at *m*/*z* 275 were observed for compound **41**, the respective signal — which should be the same if deoxygenation would occur at the NO_2 or SO_2 groups — was not found in

the MS/MS of **121** (Figure 2, B). Instead, a signal was registered at *m*/*z* 259, which is consistent with an amine instead of the hydroxylamine functionality. The fact that no ion response at *m*/*z* 275 was observed also revealed that deoxygenation of NO_2 to NO, as observed for aromatic nitro compounds,[1] was too slow to compete with the deoxygenation of the hydroxylamines. The eliminations of H_2 (formation of **122**) and of H_2O (formation of **123**) from sample compound **41** also occurred with the N-OH functional group rather than with the other groups contained in the molecule. Analogously to **121**, the MS/MS of artifacts **122** and **123** showed no ion signals at *m*/*z* 275 but signals at *m*/*z* 273 and *m*/*z* 257, respectively, which are diagnostic for decomposition located in the "left-part" of the molecules (Figure 2, C and D).

1.1. Dependence of the APCI Decomposition of Hydroxylamines on the Sample Concentration

The online coupling of HPLC to MS allows fast acquisition of MS data of an analyte directly after column chromatography. Thus, mass spectra of differently concentrated analyte solutions can be measured when reasonably broad chromatographic peaks are obtained. LC-APCI-MS of **41** (1 μg) afforded a chromatographic peak sufficiently broad to allow its splitting into several segments of 0.1 min, which each is characterized by a different analyte concentration. The averaged analyte concentration of the segments was estimated on the basis of the relative segment areas (Figure 3). The MS of the two highlighted segments with low analyte concentration, at the beginning and at the end of the peak (approx. 1.8 and 0.8 μM), showed rather high degrees of sample decomposition (r = 0.86 @ t_R 20.6–20.7 min and r = 0.90 @ 21.7–21.8 min, respectively). Still prominent but significantly less sample decomposition was observed at the time segment taken at the peak maximum (r = 0.65 @ t_R 21.0–21.1 min). Thus, less decomposition was observed when more highly concentrated solutions were investigated. This effect is general as it was also observed in other measurements, e.g., in those performed with natural samples of polyamine spider toxins. These results suggest, in accordance with previously described studies, that APCI decompositions are

surface-supported processes, which are controlled in their extent by the limited surface of the APCI interface.[8]

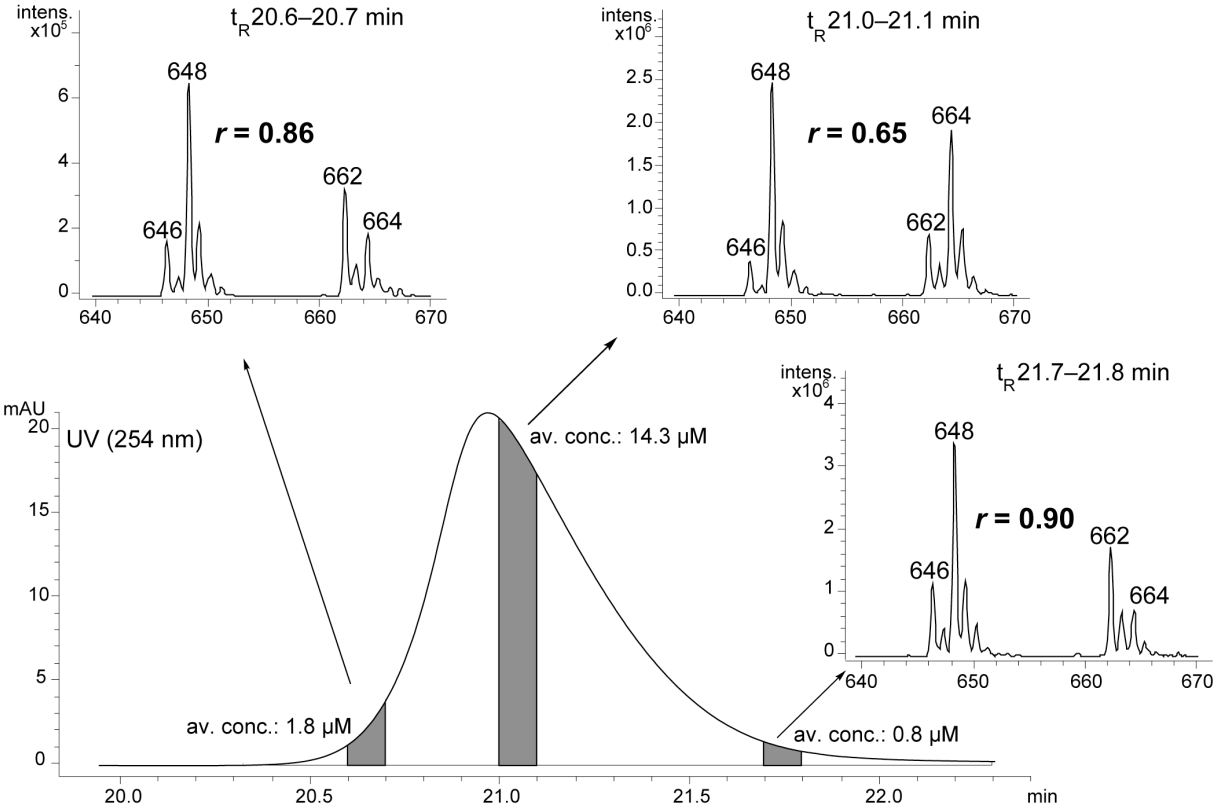

Figure 3. Concentration dependence of the APCI reduction of hydroxylamines with segments of a LC peak of **41**.

1.2. Dependence of the APCI Decomposition of Hydroxylamines on the Solvent Acidity

To study the pH dependence of the APCI decomposition of *N*-hydroxylated secondary amines, compound **41** was dissolved either in pure MeCN/H_2O (1:1) or in MeCN/H_2O (1:1) admixed with TFA, HCOOH, or TFA followed by NH_3 until neutralized and directly introduced into the APCI-MS. The results are shown with the spectra in Figure 4. Increased acidity of the sample solution led to more pronounced decomposition. While almost no decomposition of compound **41** was observed when the sample was introduced into the APCI-MS dissolved in the neutral mixture of MeCN/H_2O (1:1) (r = 0.1, Figure 4, A), the decomposition of **41** increased markedly in presence of

HCOOH or TFA (r = 0.38 and 0.54, Figure 4, B and C, respectively). Decomposition was highest when 0.1% TFA was used as the additive and therefore under the standard conditions used for the chromatographic separation of spider toxins. Decomposition of **41** was inhibited by the addition of aqueous NH_3 (to the initially acidic solution) as is recognized from spectrum D (r = 0.04, Figure 4, D). This latter result confirms the fact that the decomposition of **41** occurs in the ion source and not already before its entry into the HPLC.

The pH-dependence of the APCI decomposition of hydroxylamines can be used as a means to identify the N-OH functionality of a molecule. If a compound shows $[M+H-H_2]^+$ and, particularly, $[M+H-O]^+$ signals in APCI-MS spectra ($[M+H-H_2O]^+$ and if the formation of these ions can be enforced or inhibited by the addition of acid or base to the sample solution prior to its introduction into the instrument, the presence of the N-OH group in the molecule is rather likely. It should thus be possible to distinguish between artifacts and real N-OH-containing sample compounds by inhibition of the APCI decomposition. On the other hand, if no ions of the type $[M+H-H_2]^+$ and $[M+H-O]^+$ can be found for a compound — not even when the sample solution is acidified —, an N-OH group is most likely not present in the sample molecules.

The pH-dependence of the APCI decomposition of hydroxylamines can thus be used for the unequivocal recognition of the N-OH functionality as is illustrated below with the analysis of native polyamine toxins found in spider venom and of mayfoline, a natural cyclic polyamine derivative.[16]

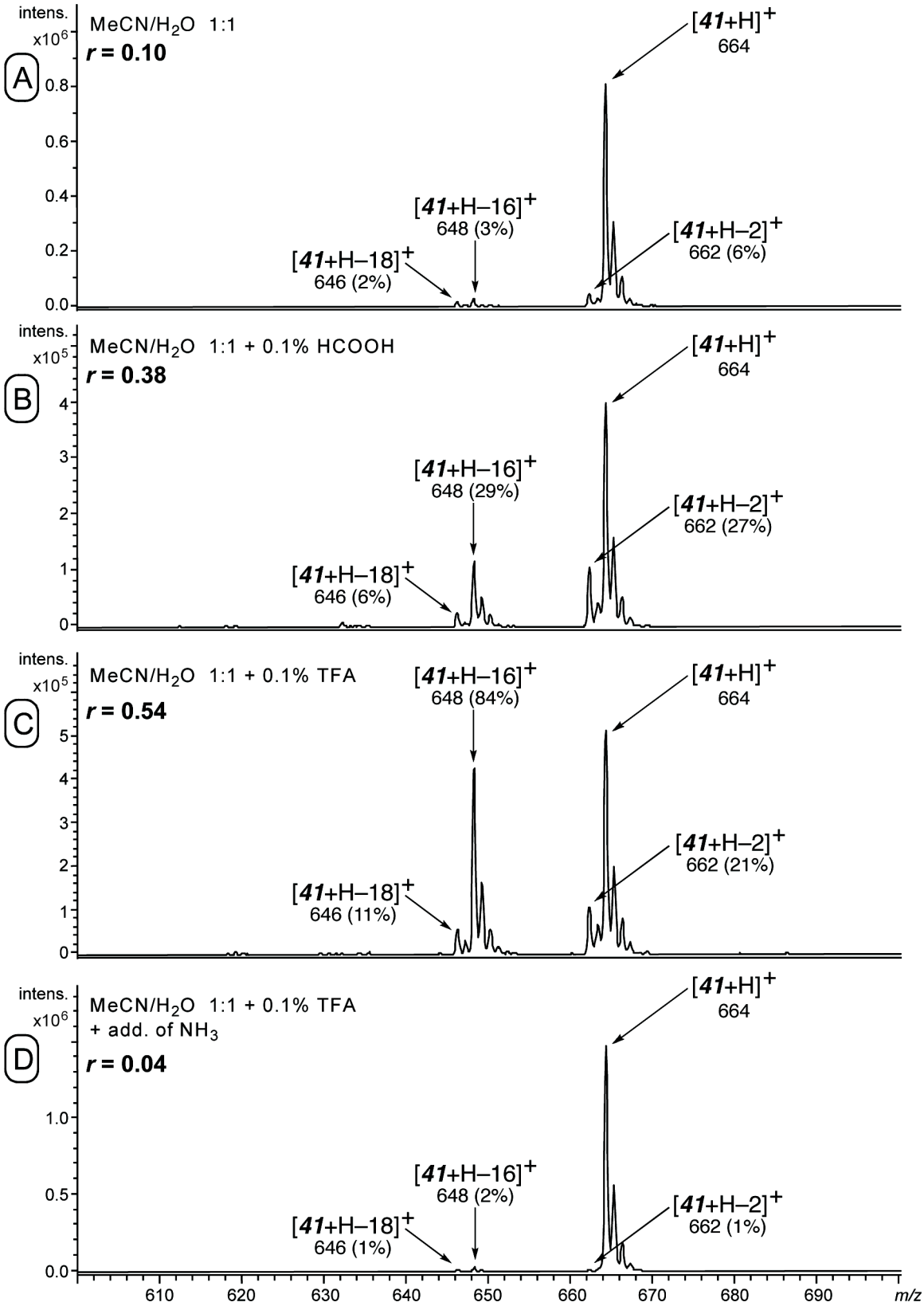

Figure 4. Direct infusion APCI-MS experiments performed with compound **41** dissolved (A) in pure MeCN/H_2O (1:1), or in MeCN/H_2O (1:1) admixed with (B) HCOOH, (C) TFA, or (D) TFA followed by addition of NH_3 until neutralized.

2. LC-APCI-MS Analysis of the Venom from the Spider *Agelenopsis aperta*

Some years ago, acylpolyamines of the venoms of Agelenopsis aperta[10] and of Paracoelotes birulai[9] were characterized by means of LC-APCI-MS and -MS/MS, and both venoms were found to contain N-hydroxylated polyamine derivatives. Due to incomplete data, some of the constituents of Agelenopsis aperta were not amenable to complete structural elucidation. They were regarded as structural isomers of other identified components, but now, with the knowledge about the in-source decomposition of N-hydroxylated secondary amines, they could also have represented artifacts. Thus, the venom of Agelenopsis aperta was reinvestigated by LC-APCI-MS.

The 2D-plot in Figure 5A summarizes the ion responses of all constituents of the acylpolyamine fraction of *Agelenopsis aperta* obtained with the LC-MS setup already used previously. The spots in the chromatogram represent ion responses registered in dependence on retention times (abscissa) and *m*/*z* values (ordinate). Remarkable is the occurrence of signal doublets and triplets characterized by the same t_R and by *m*/*z* values differing by 16 u. Interpretation of the MS/MS data of the respective ions revealed that the triplets represent ions of di-, mono-, and non-*N*-hydroxylated polyamine derivatives and the doublets of mono- and non-*N*-hydroxylated polyamine derivatives, in each case sharing the polyamine backbones. Applying the acquired knowledge of the APCI reduction of hydroxylamines, the signals of the "deoxygenated" structures were most likely due to APCI artifacts rather than the response of real sample compounds. For example, the two ion signals at *m*/*z* *449* and *433* of the doublet **B** at t_R = 25.9 min can be interpreted as the protonated molecules and the respective protonated "deoxygenated" decomposition products $[M+H-O]^+$ of the co-eluting isomeric mono-*N*-hydroxylated toxins **127 + 128**.[10] The signal at *m*/*z* 433 could therefore be generated by partial reduction of **127 + 128** during the APCI process (Figure 5, A).

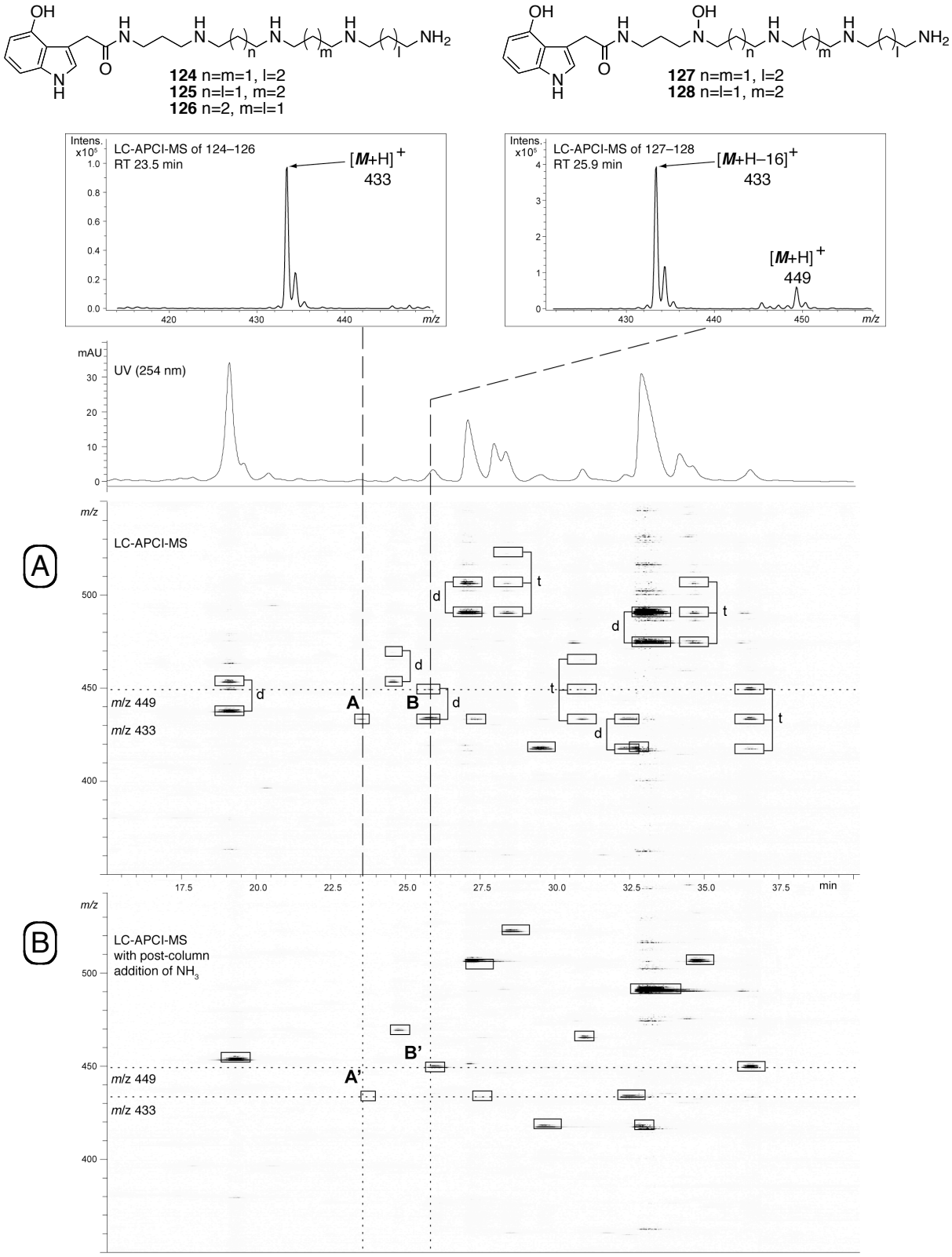

Figure 5. 2D-plot of an LC-APCI-MS run of *Agelenopsis aperta* venom and the corresponding UV-chromatogram detected at λ = 254 nm using (**a**) MeCN/H_2O + 0.1% TFA as the mobile phase and (**b**) the same conditions but with post-column addition of NH_3. d designates a signal doublet and t a signal triplet.

To prove that the doublets and triplets recorded in the 2D-chromatogram arise in fact from mono- or di-*N*-hydroxylated parent compounds, an LC-APCI-MS experiment of the toxin mixture was performed under the same optimized chromatographic conditions used before (MeCN/H_2O gradient + 0.1% TFA), but with post-column addition of NH_3 to inhibit the APCI decompositions. The respective 2D-plot is shown in Figure 5B. It is readily recognized that the doublets and triplets found in Figure 5A largely disappeared, which allows the conclusion that the several vanished peaks arose from artifacts rather than from native compounds.

Hence, also *N*-hydroxylated acylpolyamines of spider venom underwent in-source decomposition during LC-APCI-MS experiments analogously to the synthetic *N*-hydroxylated compound **41**. The re-analysis of the venom of *Agelenopsis aperta* revealed that compounds generated by APCI-decomposition were previously misinterpreted as constituents of the venom.

The eye-catching doublet- and triplet-patterns recognized in the 2D-chromatogram (A) in Figure 5 can be taken as a count for the number of N-OH groups in a molecule. Thus, two 2D-plots of *N*-hydroxylated (or potentially *N*-hydroxylated) compounds measured under acidic and basic APCI conditions can reveal the presence and number of N-OH groups in a unknown compound. Since *C*-hydroxy groups are not reduced during APCI-MS, this ionization mode can be used to distinguish *N*- from *C*-hydroxylation as shown, for instance, with the APCI-MS of the non-*N*-hydroxylated derivatives **124–126** that did not show signals for deoxygenated products. These results are in accordance with those obtained with the method reported to differentiate *N*-oxides from *C*-hydroxylated compounds.[3]

3. APCI-MS Analysis of Mayfoline

Mayfoline (**129)** (Figure 6) is a cyclic *N*-hydroxylated spermidine alkaloid isolated from the shrub *Maytenus buxifolia*,[16] and it was synthesized some years ago by *Hesse* et al.[17] Due to the N-OH functionality, Mayfoline was expected to

show the same type of APCI decomposition as the model compound **41** and the polyamine spider toxins described above. However, only a little decomposition was observed (r = 0.21, Figure 6, A) when a sample of the natural product was analyzed by LC-APCI-MS under the normal conditions applied for the separation of polyamine derivatives (MeCN/H_2O grad. + 0.1% TFA). The expected signals for the protonated decomposition products, [(***129***+H–H_2]$^+$, [(***129***+H–O]$^+$, and [(***129***+H–H_2O]$^+$, were still observed but with unexpected low relative intensities of 7%, 12%, and 8% despite the high acidity of the solvent mixture used for the APCI-MS experiment. When a sample of mayfoline was brought into the ion source in neutral solvent (MeCN/H_2O 1:1), a spectrum with only a single signal, the [(***129***+H]$^+$, was recorded. Thus, mayfoline represents an *N*-hydroxylated compound with little tendency to undergo APCI reduction.

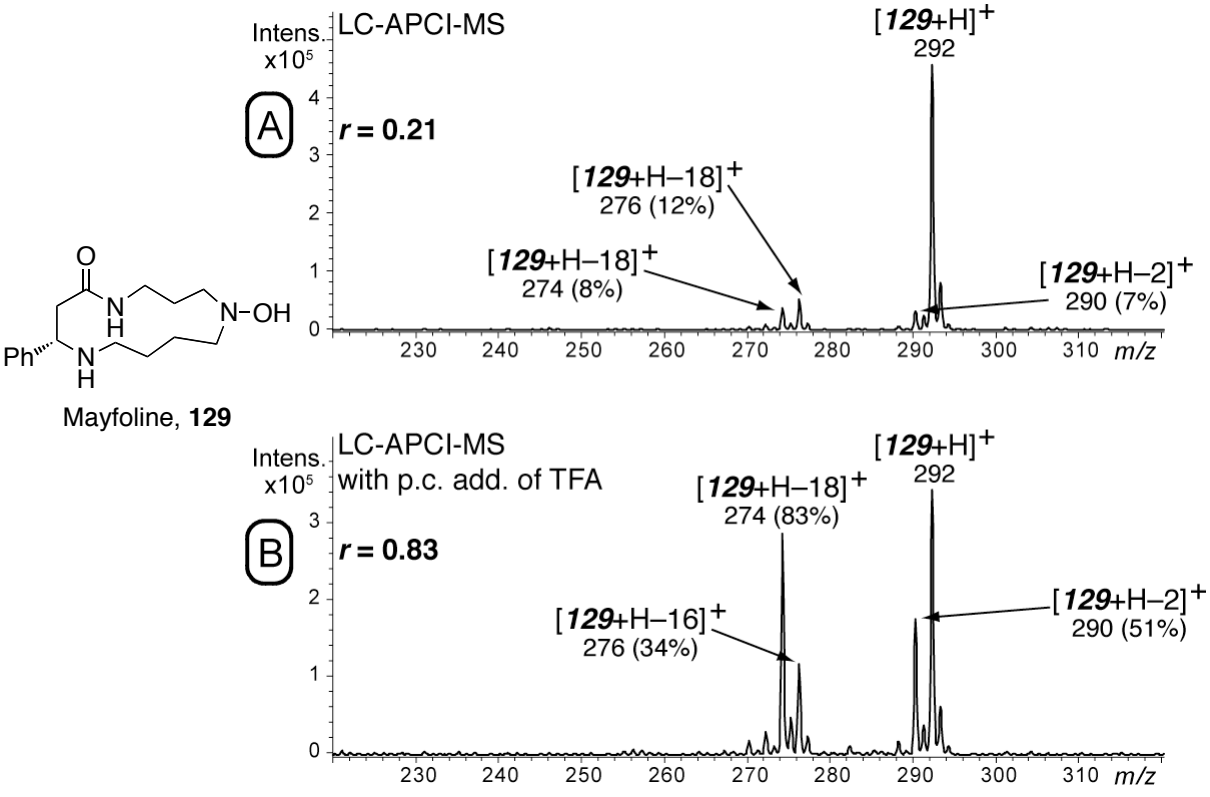

Figure 6. LC-APCI-MS of mayfoline (A) with MeCN/H_2O grad. + 0.1% TFA @ 180 µl/min and (B) with MeCN/H_2O grad. + 0.1% TFA @ 180 µl/min and post-column addition of MeCN/H_2O/TFA (2:6:2, 20 µl/min).

Since APCI deoxygenation was intended to be taken as a conclusive argument for the identification of the N-OH functionality within a sample molecule, it was tested if APCI deoxygenation of mayfoline can be enforced so that the respective ions are unquestionably recognized. This, in fact, could be affected by the post-column addition of a mixture of MeCN/H_2O/TFA (2:6:2) to the analyte flow. Under these conditions, the MS revealed signals of the decomposition products at *m*/*z* 290 ($[M+H-H_2]^+$, 51%), *m*/*z* 276 ($[M+H-O]^+$, 34%), and *m*/*z* 274 ($[M+H-H_2O]^+$, 83%) with significantly higher intensities (r = 0.63, Figure 6, B). No decomposition at all was observed with a sample of synthetic deoxymayfoline[17] treated the same way (data not shown).

4. Conclusions

The investigation of the various *N*-hydroxylated amines above revealed that N-OH-containing compounds characteristically form artifacts upon APCI. The corresponding decomposition reactions are strongly pH-, and, to a lesser degree, concentration-dependent. The degree of decomposition also depends on the exact molecular structures of the analytes. For all compounds investigated, APCI decomposition could be enforced by the addition of sufficient acid to the analyte solution and suppressed by the addition of base.

Understanding in-source decomposition of *N*-hydroxylated amines can help avoid misinterpretation of MS data from samples that contain *N*-hydroxylated analytes (particularly of LC-MS data for which no additional analytic information is available). The pH-dependence of the APCI decomposition can be applied in two ways: it can be used (1) to distinguish unavoidable artifacts from native compounds, as shown with the investigation of the spider venom of *Agelenopsis aperta*, and (2) for the conclusive identification of N-OH functionalities within a compound. The insights gained with the MS investigation of hydroxylamines are of relevance for synthetic organic chemistry as well. For instance, decompositions of hydroxylamines, prepared by *Cope* elimination of *N*-oxides, were largely suppressed by the addition of base to the reaction medium (see Chapter 4, 2.).

5. EXPERIMENTAL SECTION

5.1. Chemicals and Sample Preparation

HPLC supra grade acetonitrile (MeCN) was purchased from *Scharlau* (Barcelona, Spain), trifluoroacetic acid (TFA) and formic acid (HCOOH) from *Fluka* (Buchs, Switzerland), and aqueous solution of NH_3 (25%) from *Merck* (Darmstadt, Germany) in the respective highest qualities. HPLC grade H_2O (≤ 5 ppb total organic content) was obtained by purification of deionized H_2O with a MilliQ gradient apparatus (*Millipore*, Milford, MA, USA). [4-Hydroxy-9-(2-nitrophenylsulfonyl)-4,9-diazadodecane]-1,12-diphthalimide (**41**) was synthesized on solid support and purified by preparative HPLC.[13] Synthetic (–)-(2S)-9-hydroxy-2-phenyl-1,5,9-triazacyclotridecan-4-one (= mayfoline) was obtained from *Hesse*.[17] Lyophilized *Agelenopsis aperta* venom was purchased from Fauna Laboratories Ltd. (Almaty, Kazakhstan).

5.2. High Performance Liquid Chromatography and Mass Spectrometry

General: LC-MS analyses were performed on a *Hewlett-Packard* 1100 HPLC system (*Hewlett-Packard Co.*, Palo Alto, CA, USA) fitted with a HTS PAL autosampler (*CTC Analytics*, Zwingen, Switzerland), a *Hewlett-Packard* 1100 binary pump, and a *Hewlett-Packard* 1100 diode array detector (DAD). The reversed-phase column used was an *Interchim* Uptisphere RP C18 column (UP3HDO-20QS, 3 μm, 2.3 × 200 mm, *Interchim*, Montluçon, France). Either a step gradient or isocratic conditions at flow rates between 150 and 180 μl/min were applied with solvents A and B (solvent A: H_2O + 0.1% TFA, solvent B: MeCN + 0.1% TFA).

The LC system was connected to an EsquireLC quadrupole ion trap mass spectrometer (*Bruker Daltonik GmbH*, Bremen, Germany), equipped with either an ESI or APCI *Hewlett-Packard* Atmospheric Pressure Ion (API) source. Conditions for ESI: nebulizer gas (N_2, 40 psi), dry gas (N_2, 9 l/min), dry temperature (300 °C), HV capillary (4500 V), HV EndPlate (–600 V).

Conditions for APCI: nebulizer gas (N_2, 21 psi), dry gas (N_2, 7 l/min), dry temperature (300 °C), APCI temperature (300 °C), HV corona (2870 V), HV capillary (3713 V), HV EndPlate (–600 V). The MS-parameters (target mass, compound stability, and trap drive) were optimized for each measurement to obtain highest ion response and minimal in-source fragmentation. The MS acquisitions were performed in positive ion mode at normal resolution (0.6 u at half peak height) and under ion charge control conditions (ICC, target: 10'000). Full scan MS and MS/MS were averaged over 5 to 8 single spectra and acquired with a mass window between *m*/*z* 50 and 1000. For all MS/MS experiments, the isolation width was set to 1 Da, the fragmentation cut-off to "fast calc", and the fragmentation amplitude to 1 in the "SmartFrag" mode.

High-resolution Fourier transform (FT) mass spectral data were obtained with a LTQ-Orbitrap XL mass spectrometer (*Thermo Electron*, Bremen, Germany) equipped with a standard ESI source. Parameters: spray voltage (5 kV), tube lens voltage (120 V), capillary voltage (38 V), temperature (275 °C). The mass spectrometer was calibrated for mass accuracy immediately before each measurement according to the manufacturers instructions, the relative mass error being typically lower than 3 ppm (externally). The high-resolution FT-MS data were additionally calibrated internally during the measurements with established lock masses (*m*/*z* 429.088735 and 445.120025). Data was acquired within a mass range of *m*/*z* 150 to 1000. The AGC target setting for FT-MS experiments was set to 50'000. Spectra were acquired with a resolution of 60'000 (full width at half-maximum, FWHM) at *m*/*z* 400, and 10 spectra were averaged.

Synthetic compound (**41)**: For LC-MS analyses, 5 µl of a stock solution of **41** (200 µg) in MeCN/H_2O (1:1, 1 ml) was injected at isocratic conditions with 40% of B and a flow rate of 0.18 ml/min. Direct infusion APCI experiments were carried out by pumping 200 µl/min of a 30fold diluted stock solution of **41** into the mass spectrometer with a syringe infusion pump (*Cole-Parmer Instrument Company*, Vernon Hills, IL, USA). For FT-MS experiments, a 10fold

diluted stock solution of **41** was introduced at 6 µl/min using the same syringe infusion pump.

Spider venom: Crude lyophilized *Agelenopsis aperta* venom (100 µg) was dissolved in MeCN/H_2O (1:3, 50 µl) + 0.1% TFA, and an aliquot of 5 µl was injected into the LC-MS system. A linear gradient from 5 to 20% B over 40 min at a flow rate of 150 µl/min was applied. The post-column addition of NH_3 to the eluent was performed by the addition of an aqueous solution of NH_3 (10%) at a rate of 20 µl/min through a Tee located in-between the exit of the column and the entry of the APCI interface.

Mayfoline (**129)**: **129** (6.34 µg) was dissolved in MeCN/H_2O (1:4, 1 ml), and an aliquot of 5 µl was injected into the LC-MS system under isocratic conditions with 20% B and a flow rate of 180 µl/min. The post-column addition of TFA to the sample was performed by the addition of a mixture of MeCN/H_2O/TFA (2:6:2) at a rate of 40 µl/min through a Tee located in-between the exit of the column and the entry of the APCI interface.

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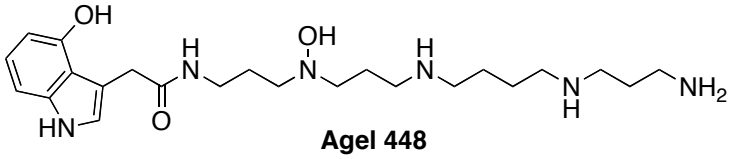
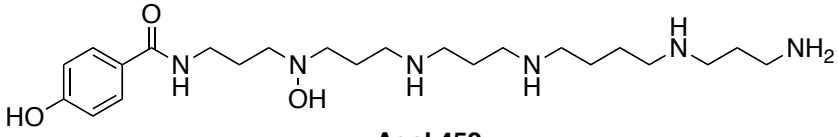
SUMMARY

The most common aliphatic polyamines, spermine, spermidine and putrescine, are ubiquitous in all living organisms. Polyamines display a wide range of biological function in microorganisms as well as plant and animal systems. They play important roles in DNA stabilisation and modification, protein biosynthesis, the modification of neuroreceptors and their associated ion channels in mammalian central nervous system. Because polyamines are considered as therapeutic leads for the treatment of a variety of brain disorders such as *Parkinson*'s and *Alzheimer*'s diseases, it is well understood why new and efficient methods for their synthesis are being sought. A number of natural products, however, are additionally derivatised at the internal N-atom, like, the *N*-hydroxylated spider toxins **Agel 448** and **Agel 452** present in the venom of the spider *Agelenopsis aperta* (Figure 1).



Agel 448



Agel 452

Figure 1. Representatives of *N*-hydroxylated polyamine spider toxins from *Agelenopsis aperta*.

The chemical composition of spider venoms is heterogeneous. Two major classes of compounds are found, complex peptides and lower molecular weight molecules such as acylpolyamines. The latters are known to provoke a fast but reversible paralysis of the prey. Our group has contributed to the elucidation of the structure of many polyamine derivatives by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and tandem mass spectrometry (MS/MS or MS^n) present in the venom of, e.g., *Agelenopsis aperta*, *Paracoelotes birulai*, *Hololena curta* and *Larinioides folium*.

N-hydroxylated polyamine derivatives were found to decompose during the ionization process of liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) experiments. Whereas liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) showed a single peak for **Agel 489**, LC-APCI-MS showed two, with a difference of 16 Da (Figure 2). This observation can be explained by the reduction of the NOH- to the NH- group. Unfortunately no literature was found to confirm or discard this hypothesis.

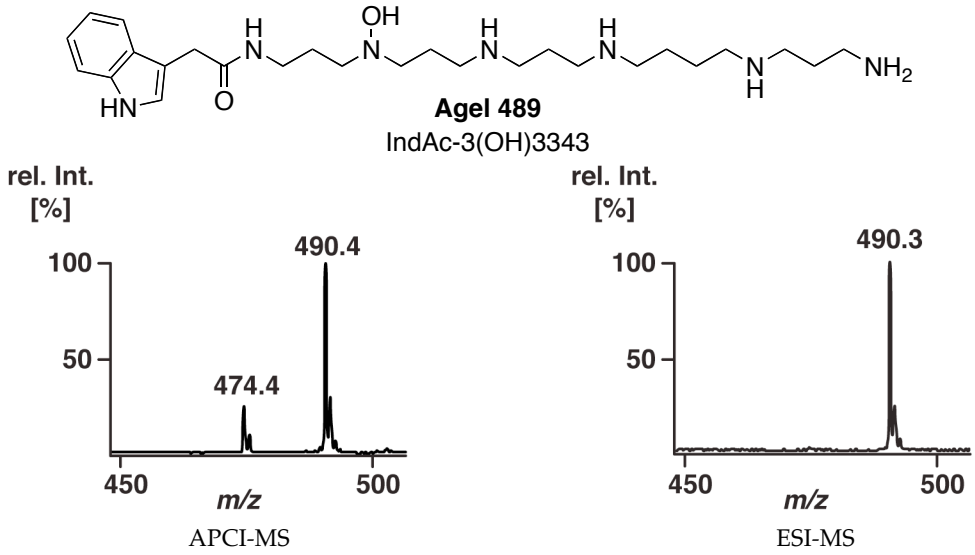
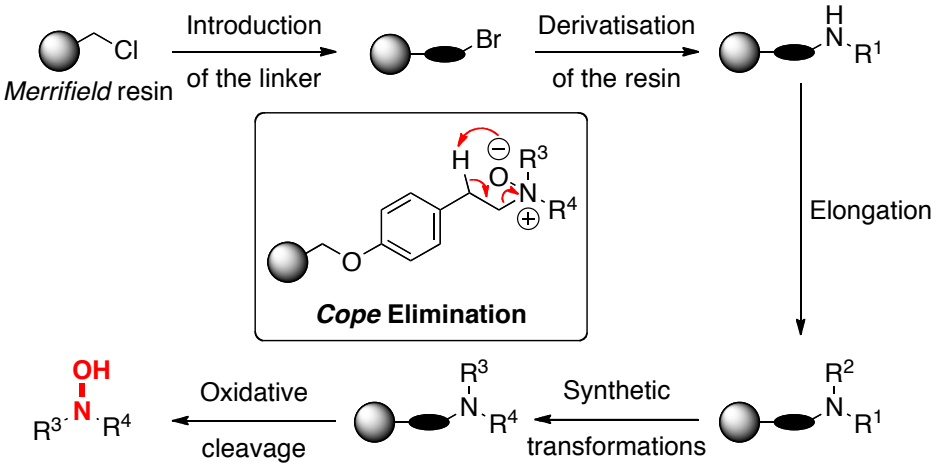


Figure 2. APCI- and ESI-MS of acyl polyamine IndAc3(OH)3343.

In connection with our ongoing studies in the analysis and synthesis of spider toxins in addition to our intention to understand the reductive behaviour during MS measurements, we were interested in extending our solid-phase strategy for the preparation of *N*-hydroxylated polyamine derivatives. Until now our method has been applied to the preparation of terminally acylated polyamine natural products and cyclic tri- and tetraamine derivatives. Poor yields obtained with the usual cleaving method, using ACE-Cl followed by hydrolysis with MeOH, led us to the introduction of a phenethyl bromide linker in-between the *Merrifield* support and the polyamine in elongation. We were confident that the *Cope* elimination provided an efficient transformation for the concurrent introduction of the desired

N-hydroxy functionality and the cleavage of the final product from the resin (Scheme 1).



Scheme 1. The concept.

This thesis presents the efficient synthesis of orthogonally protected *N*-hydroxylated linear tri-, tetra-, penta- and hexaamines on the *Merrifield* resin. It is shown that the approach for the preparation of the polyamine backbones on solid-support is flexible, allowing the contruction of the resin-bound polyamine portion by (1) reductive amination with a protected amino aldehyde (Scheme 2, orange), (2) by nucleophilic substitution with mono-protected diamines (Scheme 2, blue), with disulfonamides (Scheme 2, brown), but also (3) by reverse nucleophilic substitution with a protected aminobromide derivative (Scheme 2, orange/green) or with dibromo alkane compounds (Scheme 2, magenta). We thus laid the basis for the solid-phase synthesis of any *N*-hydroxylated polyamine derivatives.

Ns, Boc, Alloc and Phth protective groups were demonstrated to be compatible with the oxidative procedure. Selective removal of the latter protective groups and insertion of the acyl moiety were performed on solid-support in-between the oxidation and the *Cope* elimination in order to keep the regioselectivity of the oxidation under control and give access to natural products such as **Agel 395** and **Agel 432** (Figure 3).

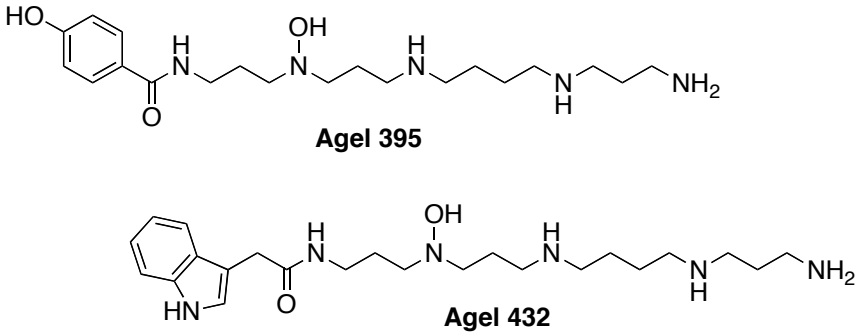
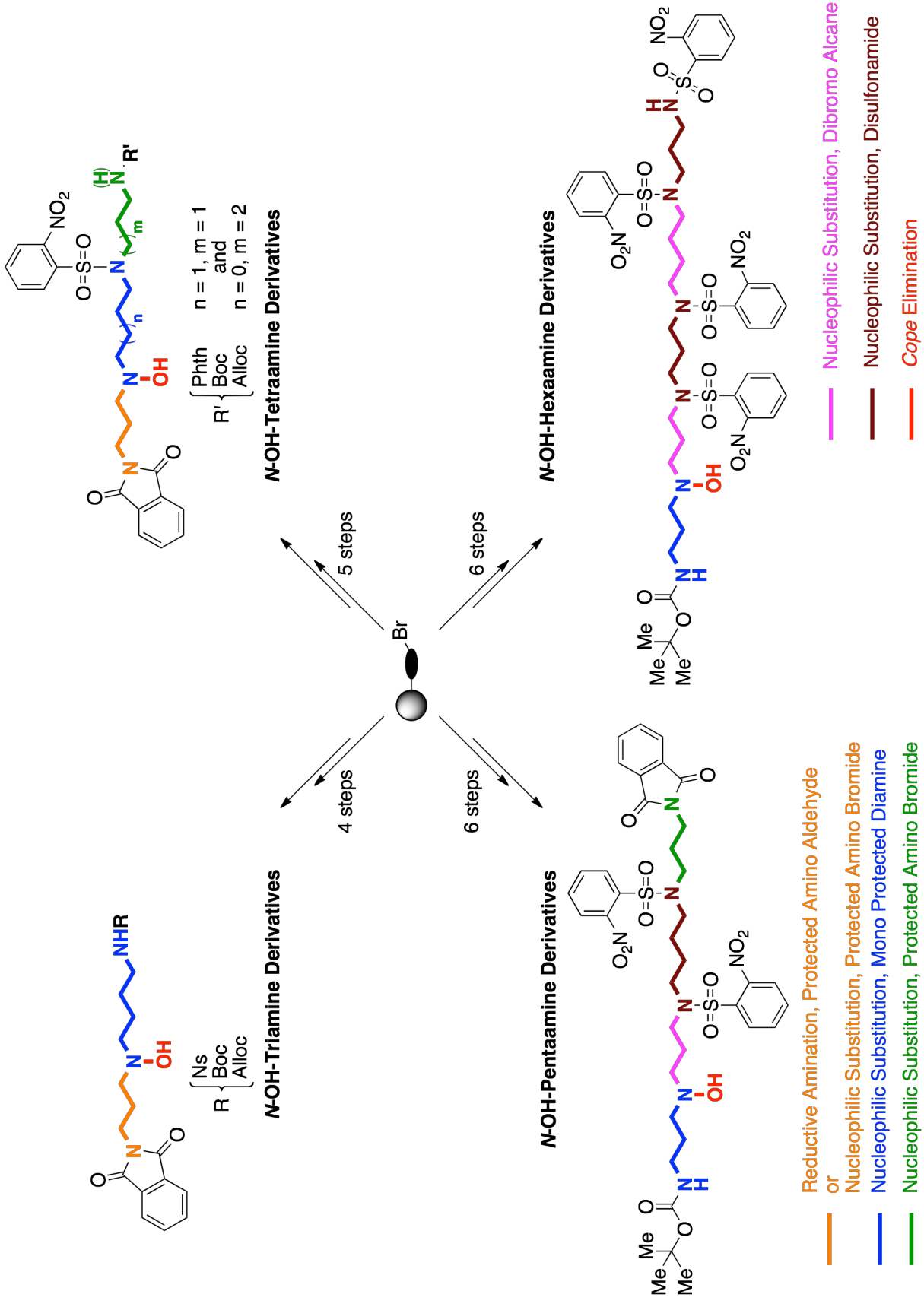


Figure 3. Two *N*-hydroxy acylpentaamine natural products.

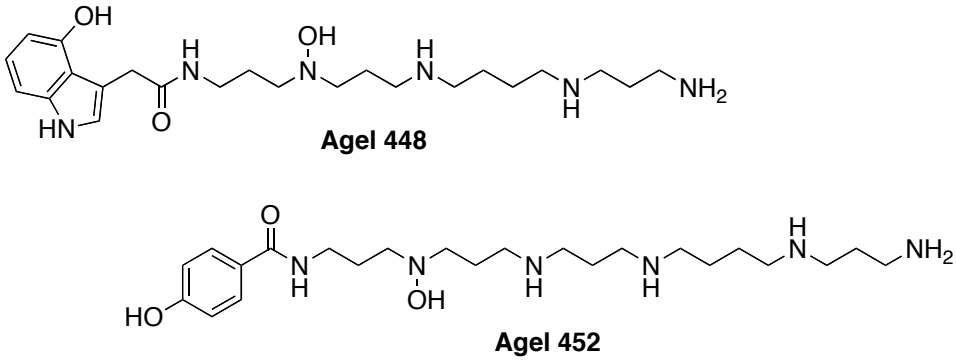
The phenomenom observed during MS measurements was studied with a model compound, a synthetic *N*-hydroxylated tetraamine derivative. It was found that the reduction, oxidation and water elimination occurred during APCI to generate the corresponding amine, *N*-oxide, and imine. The investigation further revealed that the decomposition of hydroxylamine during APCI depends upon the concentration of the analyte and on the acidity of the solution introduced into the ionization source. The pH-dependence of decomposition was utilized for the development of an MS method that allows for the unambiguous identification of N-OH fuctionalities. This method was applied for the study of natural products including polyamine toxins from the venom of the spider *Agelenopsis aperta* and mayfolin, a cyclic polyamine derivative of the shrub *Maytenus buxifolia*.



Scheme 2. The backbones preparation.

ZUSAMMENFASSUNG

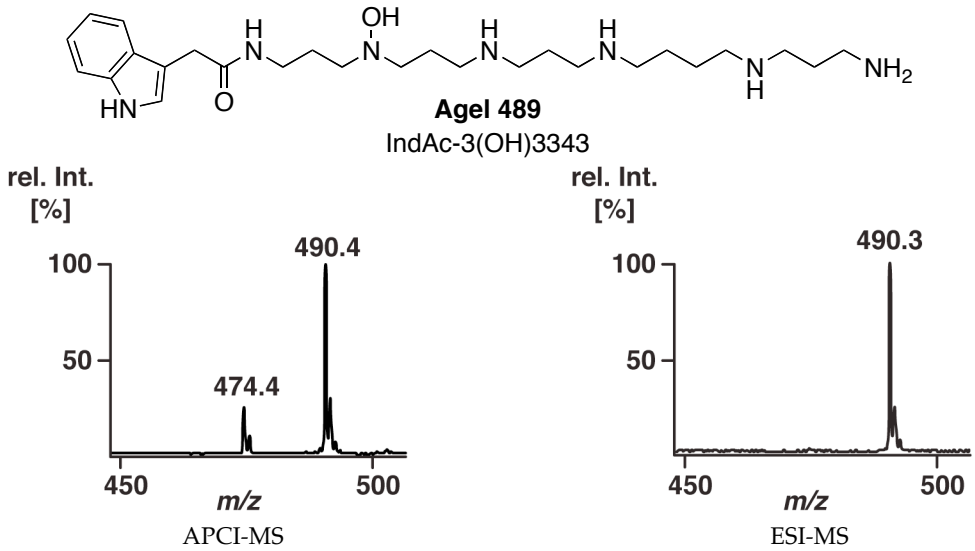
Die am weitesten verbreiteten aliphatischen Polyamine Spermin, Spermidin und Putrescin sind allgegenwärtig in lebenden Organismen. Polyamine üben eine Vielzahl von biologischen Funktionen in Mikroorganismen sowie in Pflanzen und Tieren aus. Sie tragen zur Stabilisierung und Modifizierung von DNA bei und spielen eine wichtige Rolle bei der Biosynthese von Proteinen, der Modifizierung von Neurorezeptoren und deren zugehörigen Ionenkanälen im Zentralnervensystem von Säugetieren. Da Polyamine als therapeutische Ansätze für die Behandlung von verschiedenen Hirnleistungsstörungen wie *Parkinson* oder *Alzheimer* berücksichtigt werden, ist es leicht nachvollziehbar, dass neue und effizientere Methoden für deren Synthese gesucht werden. Etliche der Naturstoffe sind an einem internen N-Atom derivatisiert, wie die *N*-hydroxylierten Spinnengifte **Agel 448** und **Agel 452** der Spinne *Agelenopsis aperta* (Figur 1).



Figur 1. Vertreter der *N*-hydroxilierten Polyamin-Spinnengifte von *Agelenopsis aperta*.

Die chemische Zusammensetzung der Spinnengifte ist heterogen. Zwei Hauptklassen von Substanzen liegen vor: komplexe Peptide und Moleküle mit niedrigem Molekulargewicht wie Acylpolyamine. Letzere sind dafür bekannt, dass sie eine schnelle, aber reversible Lähmung der Beute hervorrufen. Unsere Gruppe hat zur Aufklärung von Strukturen vieler Polyamin-Derivaten aus dem Gift von *Agelenopsis aperta*, *Paracoelotes birulai*, *Hololena curta* und *Larinioides folium* mittels HPLC-MS (high performance liquid chromatography mass spectrometry) und MS/MS (tandem mass spectrometry) beigetragen.

Es wurde beobachtet, dass *N*-hydroxylierte Polyamine in dem Ionisierungsprozess während LC-APCI-MS (liquid chromatography/ atmospheric pressure chemical ionization-mass spectrometry) Messungen zerfallen. Mittels LC-ESI-MS (liquid chromatography/electronenspray ionization-mass spectrometry) wurde für **Agel 489** wie erwartet ein Peak erhalten, wohingegen APCI-MS Analysen zwei Peaks mit einem Massenunterschied von 16 Da hervorbrachten (Figure 2). Diese Beobachtung lässt sich durch die Reduktion von NOH zu NH erklären. Allerdings wurde keine Literatur zur Stützung oder Widerlegung dieser Hypothese gefunden.

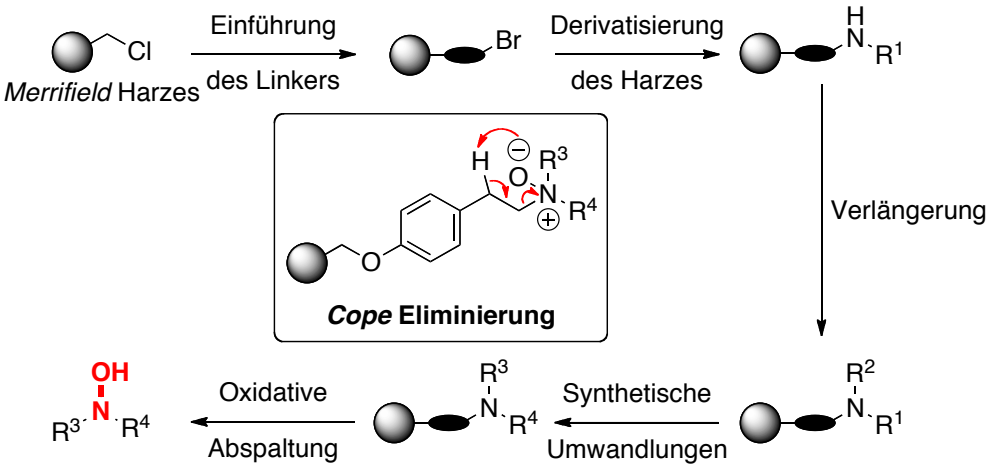


Figur 2. APCI- und ESI-MS Spektren des Acylpolyamins IndAc3(OH)3343.

Bis zu diesem Zeitpunkt wurden mit unserer Methode terminal acylierte Polyaminnaturstoffe und Cyklische Tri- und Tetraamin-Derivate synthetisiert. Zusammenhängend mit den laufenden Untersuchungen, der Analyse und Synthese der Spinnengiftkomponenten, weiteten wir unsere Strategie der Festphasen-Synthese auf *N*-hydroxylierten Polyamin-Derivate mit dem Ziel aus, das reduktive Verhalten während der MS Messungen zu verstehen.

Beim Gebrauch der üblichen Methode zur Abspaltung der Moleküle von der Festphase mit ACE-Cl, mit anschließender Methanolyse, wurden ungenügende Ausbeuten erhalten. Dies veranlasste uns zur Einführung eines Phenethyl-bromid Linkers zwischen dem *Merrifield* Harz und den zu

verlängernden Polyaminen. Wir waren zuversichtlich, dass die *Cope* Eliminierung eine effiziente Umwandlung zur gewünschten *N*-Hydroxy-Funktionalität und die gleichzeitige Abspaltung des Produktes vom Harz gewährleisten würde (Schema 1).

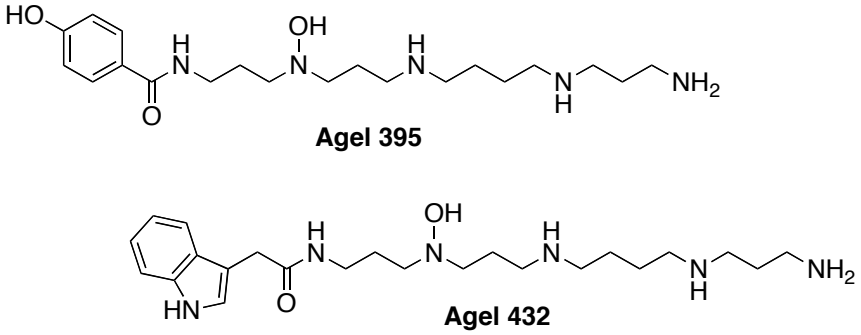


Schema 1. Das Konzept.

Diese Arbeit beschreibt eine effiziente Methode zur Herstellung von orthogonal geschützten, *N*-hydroxilierten, linearen Tri-, Tetra-, Penta- and Hexaamine am *Merrifield*-Harz. Wir zeigen, dass der Ansatz Polyamin-Strukturen auf der Festphase zu synthetisieren flexibel ist, weil er den sequentiellen Aufbau des Polyaminrückrates durch (1) reduktive Aminierung mit geschützten Aminoaldehyden (Schema 2, orange), (2) durch nukleophile Substitution mit einfach geschützten Diaminen (Schema 2, blau) oder Disulfonamiden (Schema 2, braun), (3) aber auch durch invertierte nukleophile Substitution mit geschützten Aminobromid-Derivaten (Schema 2, orange/grün) oder mittels Dibromoalkanen (Schema 2, magenta) ermöglicht. Mit dieser Arbeit wurde die Basis für die Festphasen Synthese praktisch beliebigen *N*-hydroxilierter Polyamin-Derivate gelegt.

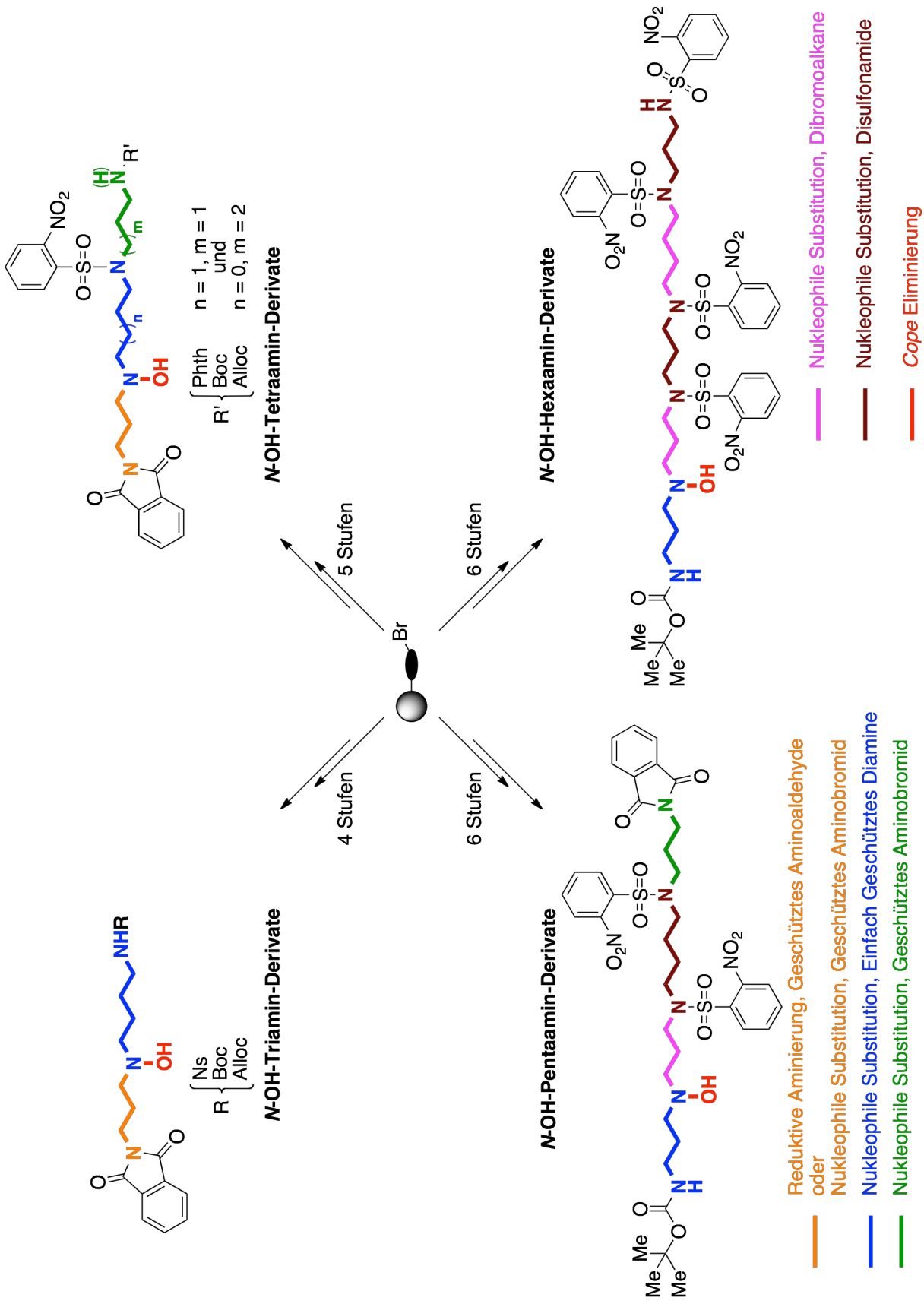
Wir haben gezeigt, dass Ns, Boc, Alloc und Phth Schutzgruppen kompatibel mit der oxidativen Prozedur der Abspaltung des Endproduktes sind. Die selektive Entfernung der genannten Schutzgruppen und die Einführung eines

Acyl-Rests wurden auf der Festphase, zwischen der Oxidation eines Amins zum entsprechenden N-Oxides und einen *Cope* Eliminierung, durchgeführt, um einerseits die Regioselektivität der Oxidation zu kontrollieren und andererseits den Zugang zu Naturstoffen wie z. B. **Agel 395** und **Agel 432** (Figur 3) zu erhalten.



Figur 3. Zwei *N*-hydroxyacylpentaamin-Naturstoffe.

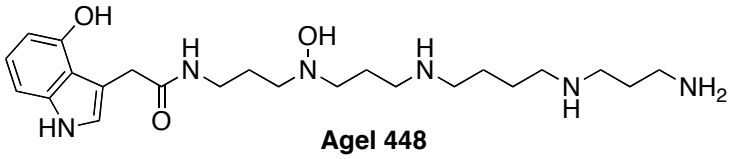
Die Untersuchung von Phänomenen, die während APCI-MS-Messungen aufgetretenen sind, wurden mittels einer synthetischen *N*-hydroxylierten Modellverbindung durchgeführt. Sie zeigten, dass bei der APCI von *N*-hydroxylierten Verbindungen Reduktion, Oxidation und Wasserelimination ablaufen und dass die entsprechenden Amine, *N*-Oxide und Imine gebildet werden. Dabei hängt die Zersetzung der Hydroxylamine von der Konzentration des Analyten sowie der Acidität der durch die Ionenquelle eingesprühten Lösung ab. Diese pH-Abhängigkeit wurde genutzt, um eine MS-Methode zu entwickeln, welche die eindeutige Identifikation von N-OH Funktionalitäten erlaubt. Diese Methode wurde für weitere Studien an Naturstoffen wie den Polyaminen des Spinnengiftes von *Agelenopsis aperta* und dem Cyklischen Spermidin-Alkaloid Mayfolin der Pflanze *Mayetenus bruxifolia* angewandt.



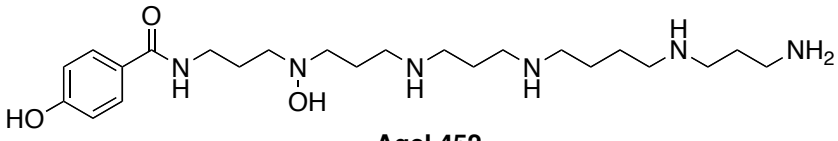
Schema 2. Synthese des Polyamingerüsts.

RÉSUMÉ

Les polyamines les plus courantes, spermine, spermidine et putrescine, sont omniprésentes dans tout organisme vivant. Les polyamines possèdent une large gamme de fonctions biologiques aussi bien dans les systèmes végétaux qu'animaux. Elles jouent d'importants rôles aux niveaux de la stabilisation et la modification de l'ADN, mais aussi dans la modification de certains neurorécepteurs ainsi que leurs canaux ioniques associés dans le système nerveux central des mammifères. Parce que les polyamines sont considérées comme de prometteurs candidats thérapeutiques dans le traitement de certaines affections du cerveau comme les maladies de *Parkinson* et d'*Alzheimer*, nous comprenons pourquoi la mise au point de nouvelles et efficaces méthodes pour leur synthèse sont étudiées. Cependant, un certain nombre de produits naturels se trouvent être dérivés à l'un des atomes d'azote internes. C'est le cas des toxines *N*-hydroxylées **Agel 448** et **Agel 452** présentes dans le venin de l'araignée *Agelenopsis aperta* (Figure 1).



Agel 448



Agel 452

Figure 1. Representations de polyamines *N*-hydroxylées, toxines d'*Agelenopsis aperta*.

La composition du venin d'araignée est un mélange hétérogène. Deux catégories majeures de produits sont rencontrées, de complexes peptides et des composants de masses moléculaires plus faibles, les polyamines acylées. Ces dernières sont réputées pour provoquer de rapides, mais réversibles, paralysies des proies. Notre groupe a contribué à la détermination de la structure de nombreux dérivés polyaminés par HPLC-MS («high-performance liquid chromatography» couplée à la spectrométrie de masse) et MS/MS (ou

MS^n, successives spectrométries de masse) présents dans le venin de certaines araignées, comme par exemple, *Agelenopsis aperta*, *Paracoelotes birulai*, *Hololena curta* et *Larinioides folium*. Il a été observé que les dérivés polyaminés *N*-hydroxylés décomposent pendant le processus d'ionisation lors des expériences de LC-APCI-MS («liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry»). Tandis que les mesures par LC-ESI-MS («liquid chromatrography-electrospray ionization-mass spectrometry») montraient une valeur unique pour **Agel 489**, les mesures par APCI-MS en montraient deux avec une différence de 16 Da (Figure 2). Cette observation peut être expliquée par la réduction d'un groupe NOH- en NH-, malheureusement aucun cas similaire ne fût rapporté dans la littérature pour confirmer ou écarter cette hypothèse.

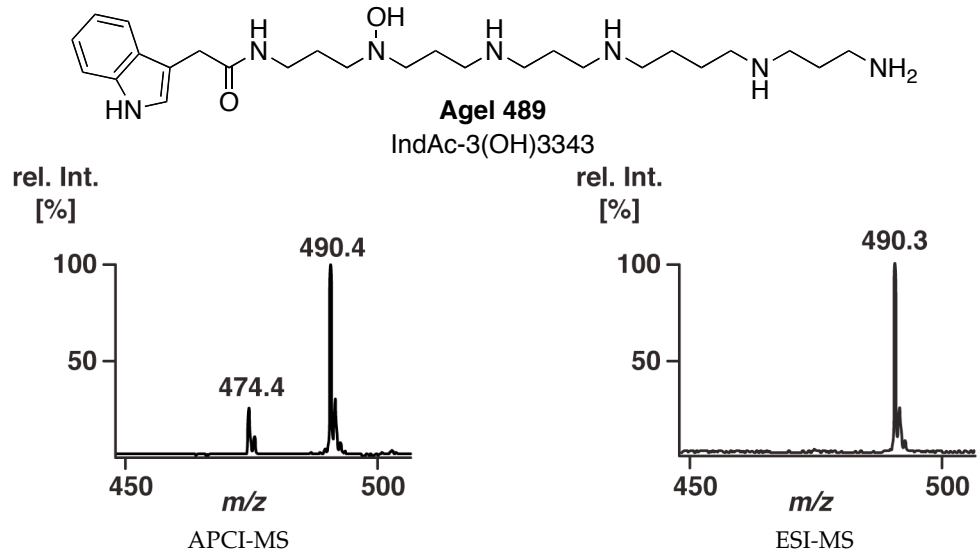


Figure 2. APCI- et ESI-MS de l'acylpolyamine IndAc3(OH)3343.

En rapport avec nos études actuelles concernant l'analyse et la synthèse des toxines d'araignées ainsi que notre intention de comprendre ce mécanisme de réduction pendant les mesures de spectrométrie de masse, nous nous sommes intéressés à l'extention de notre stratégie de synthèse sur support solide à la préparation de dérivés polyaminés *N*-hydroxylés. Jusqu'à présent, notre méthode avait été appliquée à la préparation de polyamines avec une fonction acyl terminales (produits naturels) ainsi qu'à la synthèse de dérivés tri- et tetraaminés cycliques. Les faibles rendements obtenus avec la méthode habituelle de clivage, utilisant ACE-Cl suivi par une hydrolyse au méthanol, nous

ont conduit à introduire un « phenethyl bromide linker » entre la résine de *Merrifield* et la polyamine en préparation. Nous étions confiants que l'élimination de *Cope* nous fournirait une transformation efficace pour l'introduction de la fonction *N*-hydroxy désirée simultanément au clivage du produit final de la résine (Schéma 1).

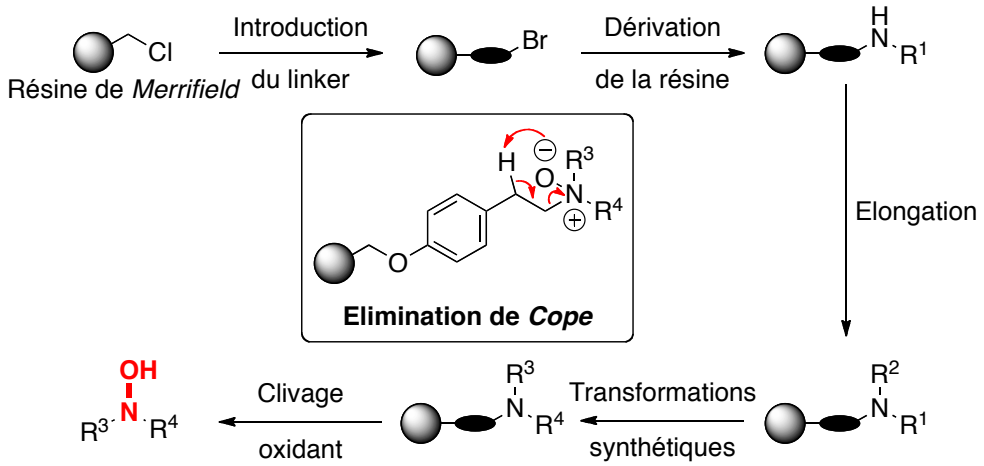


Schéma 1. Le concept.

La présente thèse expose la synthèse efficiente de dérivés *N*-hydroxylés tri-, tetra-, penta- et hexaaminés linéaires, orthogonalement protégés sur la résine de *Merrifield*. Il est démontré que l'approche utilisée pour la préparation du des dérivés polyaminés sur support solide est flexible. L'allongement du squelette peut être effectué de différentes manières: (1) par amination réductrice d'un amino-aldéhyde protégé (Schéma 2, orange), (2) par substitution nucléophile à l'aide de diamines mono-protégées (Schéma 2, bleu) ou de disulfonamides (Schéma 2, marron), mais aussi (3) par substitution nucléophile "inverse" d'un composé amino-bromé protégé (Schéma 2, orange/vert) ou d'un dibromo-alcane (Schéma 2, rose). Nous avons ainsi étendu les bases de la synthèse sur support solide de polyamines à tout dérivés *N*-hydroxylés.

La compatibilité des groupes protecteurs Ns, Boc, Alloc et Phth avec la procédure de clivage oxidant a été démontrée. La suppression de ces derniers groupes protecteurs et l'attachement de la tête acylée ont été réalisés sur support solide entre les étapes d'oxydation et d'élimination de *Cope* dans le

but de garantir la régiosélectivité de l'oxydation et d'avoir accès aux produits naturels, en particulier **Agel 395** et **Agel 432** (Figure 3).

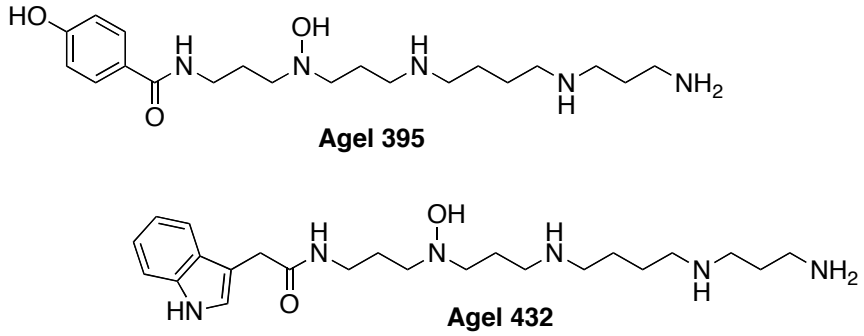


Figure 3. Deux naturelles *N*-hydroxy acylpentaamines.

Le phénomène observé lors de mesures de spectrométrie de masse a été étudié à l'aide d'un produit modèle, un dérivé tétraaminé *N*-hydroxylé synthétique. Il a été découvert que la réduction, l'oxydation ainsi que l'élimination d'eau prennent place pendant l'APCI pour générer les correspondants amine, *N*-oxide et imine. Des études plus poussées ont révélé que la décomposition des amines hydroxylées dépend de la concentration du composé lui-même mais aussi de l'acidité de la solution introduite dans la source d'ionisation. La question de la dépendence au pH a été utilisée pour le développement d'une méthode de spectrométrie de masse qui permet l'identification sans ambiguité de la fonction N-OH. Cette méthode fut appliquée à l'étude de produits naturels comme les toxines polyaminées du venin de l'araignée *Agelenopsis aperta* ou comme la mayfoline, une polyamine *N*-hydroxylée cyclique de l'arbuste *Maytenus bruxufolia*.

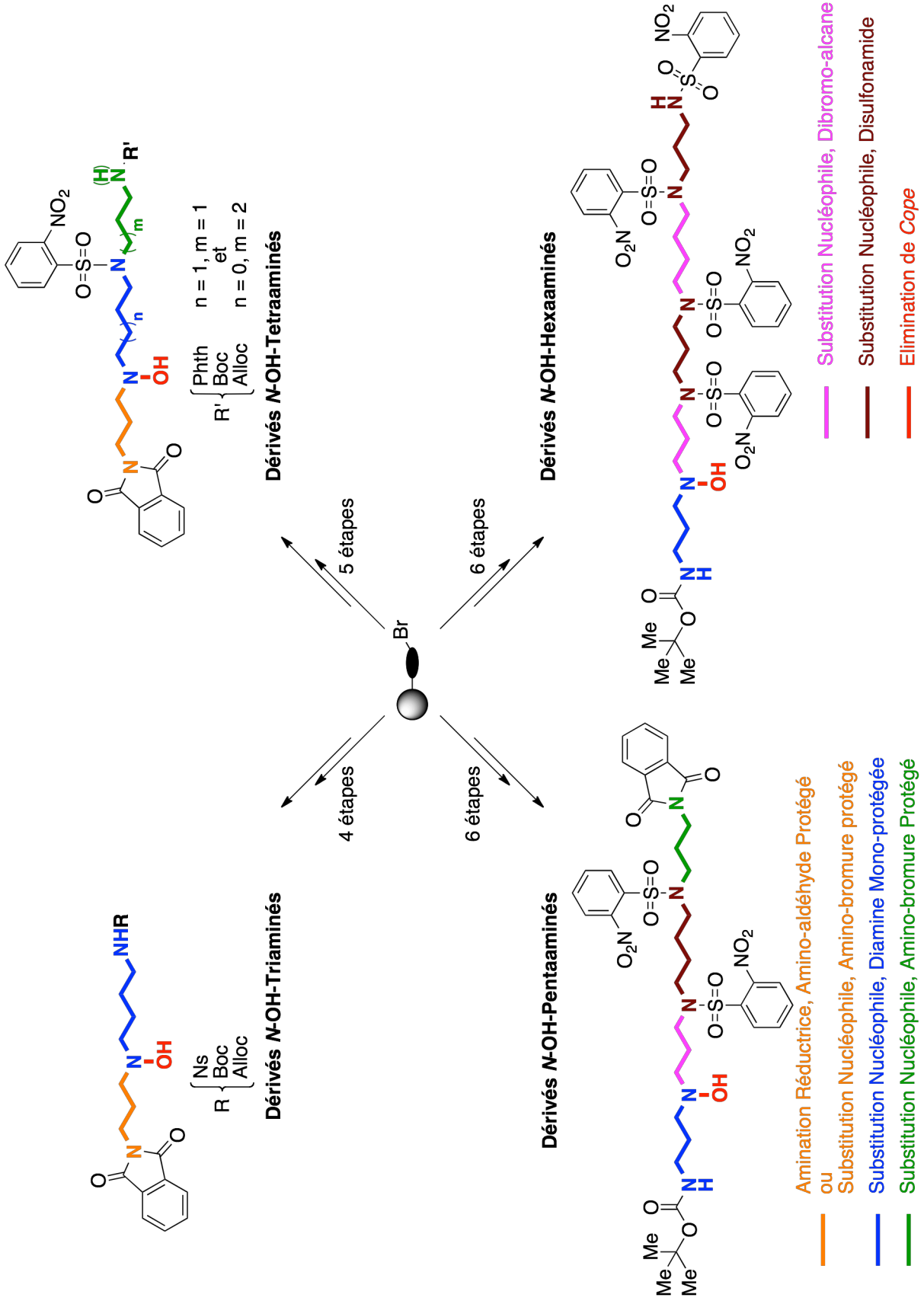


Schéma 2. Préparation des squelettes.

LIST OF ABBREVIATIONS

Ac	acetyl
ACE-Cl	1-chloroethyl chloroformate
Alloc	allyloxycarbonyl
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors
ap.	apparently
arom.	aromatic
APCI	atmospheric pressure chemical ionisation
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
CTrR	2-chlorotrityl resin
calcd.	calculated
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	diethyl azodicarboxylate
DEPT	distorsionless enhanced by polarisation transfer
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DNA	deoxyribonucleic acid
DMF	*N*,*N*-dimethylformamide
ESI	electrospray ionisation
Fmoc	9-fluorenylmethoxycarbonyl
FT-IR	*Fourier* transform infra red
gem	geminal
HPLC	high performance liquid chromatography

HR-MS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
IndAc	indolacetic acid
IR	infra red
m-CPBA	*meta*-chloroperbenzoic acid
Mp	melting point
MS	mass spectrometry
NMP	1-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
Ns	2-nitrophenylsulfonyl
o	*ortho*
2,5-$(OH)_2$-Bz	2,5-dihydroxybenzoic acid
4-OH-Bz	4-hydroxybenzoic acid
4-OH-IndAc	4-hydroxyindolacetic acid
PA	polyamine
p	*para*
p. c. add.	Post column addition
Phth	phthaloyl
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
TFA	trifluoroacetic acide
Tfa	trifluoroacetyl
TLC	thin layer chromatography
TSP	trimethylsilyl-2,2,3,3-tetradeuteropropionic acid
TrR	chlorotrityl resin
vic	vicinal

CURRICULUM VITAE

Personal Data

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Education

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1999–2001	**University Pierre et Marie Curie, Paris VI**, France — DEUG in Life Sciences
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2002–2003	**University Pierre et Marie Curie, Paris VI**, France — Maîtrise in Chemistry, specialised in Organic and Bioorganic Chemistry
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Experiences

2003 — 5 months	**University of Liège**, Belgium — Maîtrise thesis under the supervision of Prof. Albert Demonceau — *Olefin Cyclopropanation Catalysed by Ruthenium* N-*Heterocyclic Carbene Complexes*.
2004 — 6 months	**École Normale Supérieure**, Paris, France — DEA thesis under the supervision of Prof. Pierre Sinaÿ — *Study of Oxidative Decarboxylation on Anomeric Position of a Glucoside*.
2005–2009	**University of Zurich**, Organisch-Chemisches Institut, Switzerland — PhD thesis under the supervision of Prof. Dr. Stefan Bienz — *Development of an Efficient and Flexible Method for the Solid-Phase Synthesis of* N-*Hydroxypolyamine Derivatives*.

Publications

Efficient and Flexible Solid-Phase Synthesis of *N*-Hydroxypolyamine Derivatives. Michaël Méret and Stefan Bienz *Eur. J. Org. Chem.* **2008**, *33*, 5518–5525.

Decomposition of *N*-Hydroxylated Compounds during Atmospheric Pressure Chemical Ionization. Silvan Eichenberger, Michaël Méret, Stefan Bienz and Laurent Bigler *J. Mass Spectrom.* **2010**, *45*, *2*, 190–197.

Presentations

2008 Poster at the 2nd European Chemistry Congress in Turin, Italy. *Solid-Phase Synthesis of* N-*Hydroxypolyamine Derivatives*.

2008 Poster at the 2nd European Chemistry Congress in Turin, Italy. *Towards a New Linker for the Synthesis of* N-*Hydroxypolyamines on Solid Support*.

2008 Poster at the 2nd European Chemistry Congress in Turin, Italy. *Application of a Chiral Silyl Moiety in Aldol Reaction*.

2007 Oral presentation at the Doktorandentag of the Institute of Organic Chemistry of the University of Zurich. *Solid-Phase Synthesis of* N-*Hydroxypolyamine Derivatives*.

2006 Poster at the 1st European Chemisty Congress in Budapest, Hungary. *Solid-Phase Synthesis of* N-*Hydroxypolyamine Derivatives*.

2003 Poster at the 7th Sigma-Aldrich Organic Synthesis Meeting in Spa, Belgium. *Olefin Cyclopropanation Catalysed by Ruthenium* N-*Heterocyclic Carbene Complexes*.

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